STUDIES OF ORGANISATION IN THE LUNG WITH PARTICULAR REFERENCE TO PULMONARY INFARCTION

A THESIS PRESENTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF THE UNIVERSITY OF EDINBURGH

MAY, 1959.

by

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INTRODUCTION

A search of literature of experimental surgery and pathology on the subject of organisation and regeneration of lung tissue after injury, either by trauma or by infection, has yielded only a few relevant papers. On the experimental side, reference can be made only to the papers of Olch and Ballon (1929) and Montgomery (1943). Olch and Ballon made linear incisions in the lung of dogs at right angles to the main axis of the lobe, and reported that the wounds healed as scars which did not differ materially from scars elsewhere in the body. The source of the scar, however, was not determined. Montgomery removed triangular wedges, each side approximately 3 cm. long, in cats after exposing the lung by thoracotomy. The animals were killed at intervals between 36 hours and 115 days. He showed that under these conditions scar tissue formed through the organisation of the resultant haematoma and that new alveoli appeared in the scars. In some instances, it was clear that the alveoli/

alveoli were formed from pre-existing bronchi which penetrated the scar tissues, first as solid and then as tubular buds. Alveoli of this nature were lined by cubical cells and showed direct continuity with the bronchi. In others this relationship was not obvious and the new alveoli appeared as indentations of the scar tissue, lined only in part by cubical cells. The long-term experiments showed that in the cat, complete regeneration of lung could occur, and that only a dimple on the pleura might remain to mark the site of the excision.

On the observational side, the literature is equally scanty. Bremer (1937) discussed the problem whether true regeneration occurs in the lungs, but failed to reach a conclusion. He studied the fate of the lung tissue remaining after lobectomy or pneumonectomy and concluded that the permanent result of lobectomy or pneumonectomy might be either a simple distension of the remaining lung tissue by dilatation of individual alveolus and respiratory units, marked by the presence of tubular sprouts indicative of normal growth. He observed that regeneration occurred in the young whose lungs had/

had normal growth capacity, in adults whose lungs had ceased growing, dilatation was the rule. He remarked that in the rat and probably in other rodents in which normal growth continues almost throughout life, regeneration of the remaining lung could be expected in fully-grown animals. Bremer (1935) also called attention to the method of normal post-natal growth of lung by means of minute tubular sprouts which burrowed into the loose tissue, interlobular, peribronchial or subpleural, and these expanded into new alveoli.

Two diametrically opposed views with regards to the end result of lung tissue remaining after lobectomy or pneumonectomy can be cited. Hassler (1892), in a dog of ten weeks, found that the remaining right lung after six months of pneumonectomy filled the thorax and showed histologically normal structure. In adult animals this was not the case. Hilber (1934) stated that, in his operations on rats, an initial compensation for the loss of one or more lobes was accomplished by an increase in the level of the diaphragm, displacement of the heart, and mediastinum, often accompanied by scoliosis convex to the opposite side. There

was/

was also an increase in size of the remaining lung by simple distension of the alveoli, but, in addition, the lung was gradually replaced by true regeneration of pulmonary tissue, accomplished by new growth, entailing an increase in the number of the respiratory units with new alveoli of normal size and structure, and with remodeling of the bronchial tree. On the other hand, Rienhoff, Reichert and Heuer (1935) held the opposite view: "compensatory changes in dogs consists of simple dilatation of the respiratory lobules or the definititive respiratory units made up of the bronchiolus respiratorious, the ductus alveolaris, the atria, the small sacculi alveolares, and the alveoli. This dilatation comes in response to increased physiological demands and is of a compensatory nature. It is in no sense of the word an emphysema and there is no interruption or diminution of the elastic tissue or fusion of the alveoli to suggest pathological change in the lung parenchyma. There is no increase in the number of the bronchial tree or in their pattern, and apparently the blood vascular system, except for a possible dilatation, is unaffected. No evidence of a true hyperplastic regeneration or/

or a hypertrophy is found." These changes referred to the end result of operations performed by them on animals. Describing some of the morphological effects of experimental pneumonectomy found on examination of dogs killed at intervals of from 5 days to 8 years after operation, Behrend and Mann (1939) observed that following pneumonectomy the remaining lung enlarged and became heavier. The enlargement was due to an increase in the number of pulmonary lobules functioning at a given time or to simple dilatation of the air cells, or both. The increase in weight of the remaining lung was probably due to the added blood in this one lung and to an increase in the number of histiocytes. Microscopically, the only changes were focal areas of histiocytic proliferation with resultant thickening of the alveolar wall. In the course of years these histiocytes acquired the characteristics of fibrocytes. New capillaries were found to permeate the focal areas of histiocytic proliferation, a change which began about 6 weeks after pneumonectomy. This was possibly an important factor in the compensatory adjustment after pneumonectomy.

Mollgaard and Rovsing (1910) recognised both/

both possibilities, dilatation and hyperplasia, the latter occurring only in young animals. Bremer (1937) tried to show that either of these results is possible, the determining factor being the capacity for further growth of the remaining lung in each individual.

Cournand et al (1947) reported a follow-up study of the cardio-pulmonary function through the period of growth of four patients with pneumonectomy performed during childhood or early adolescent and observed moderate degree of distension of the lung in three patients. The fourth subject showed no evidence of pulmonary distension and his cardio-pulmonary performance was indistinguishable from that of a normal individual under the same strenuous circumstances. Simple dilatation of lobules assessed microscopically and physiologically was the only change noted in adults in similar circumstances by a number of investigators. (Drastich et al, 1933; Rienhoff et al; Longacre et al, 1937; Lester et al, 1942).

In summary, it may be said that published reports make a distinction between the behaviour of lung remaining after pneumonectomy on the basis/

basis of age, hyperplastic regeneration being a feature of young animals.

References to proliferation in association with pathological lesions of lung are equally scanty. In a series of 1450 consecutive autopsies in a general hospital, King (1954) found 15 examples of proliferation of epithelium of unusual character in the distal portion of the broncheal tree. Of the 15 cases of his series, 11 showed proliferation in relation to pulmonary infarct, extensive atelectasis, pulmonary fibrosis, or a combination of these conditions. Proliferation was seen also in 2 cases with chronic passive congestion without infarction, atelectasis or fibrosis, and in 2 cases without any of these features. King discussed the subject of this epithelial proliferation from many aspects but did not mention specifically regeneration of pulmonary epithelium. Wainwright (1958), in a series of 30 cases of giant-cell pneumonia, believed to be of viral origin, in African children saw regeneration of bronchial epithelium, sometimes with squamous metaplasia; solid buds of epithelial cells grew into the alveoli in the organised giant-cell lesion which presented the features of a chronic interstitial pneumonia./

pneumonia.

Thus, though there are some isolated references in literature, the possibility of regeneration of lung is not generally recognised. Indeed, current teaching is expressed by the statement in Maximow and Bloom's Text-Book of Histology (1957) - "There is no evidence that the pulmonary tissue can regenerate after destruction".

Many aspects of this reparative process of lung tissue after destruction require elucidation. This thesis attempts to examine some of them including the occurrence of reparative changes in infarcts of lung in human autopsy material and in lungs removed surgically in tuberculosis and bronchiectasis. This comprises Part I. Part II is devoted to the study of regeneration of tracheal epithelium in rats after curettage as an introductory exercise to the experimental study of lung repair. Studies on the regeneration of lung tissue in experimental healing wounds of lung in cats with some references to intracellular chemistry and to the uptake of radio-active sulphur by the regenerating pulmonary epithelium occupy Part III. Part IV consisted of the study of healing/

healing of experimental pulmonary infarcts in cats and rabbits. In Part V there is a discussion of the significance of these findings in relation to regeneration of lung. This part also contains the general summary of the whole work.

CELLULAR LINING OF THE PULMONARY ALVEOLI

Microscopical examination of the sections of organised infarcts of human lung obtained post-mortem, and of the experimentally produced wounds and infarcts of animal lungs, in the present series of my work, has frequently revealed areas of alveoli lined by prominent cells. The source and the functional importance of these lined alveoli have been a matter of controversy. In the circumstances, a brief review of the contributions of different workers on this problem is relevant.

The lining of the pulmonary alveoli and the origin of the mononuclear phagocytes, frequently seen in the alveolar spaces have been the object of numerous investigations and discussions. Three views have been advocated as to the existence and nature of alveolar lining cells.

(1) There exists normally a complete endodermal endothelial/

endothelial lining of the alveolar walls, continuous with that of the respiratory bronchioles. This view has the support of the majority of workers (Foot, 1927; Gardner and Smith, 1927; Young, 1928 & 1930; Miller, 1932; Bensley & Bensley, 1935; Bremer, 1935; Parker & Weiss, 1936; El Gazayerli, 1936; Ira Ross & Durham, 1939; Miller, 1947; Low & Sampaio, 1957). It agrees with the opinions of majority of embryologists (Frazer, 1940; Keith, 1948) who believe the lining cells to be endodermal in origin. (2) Other workers (Wanslaw, 1930; Brodersen, 1933; Palmer, 1936) do not accept a continuous lining and recognise only an interrupted cellular lining though they concede its endodermal origin. (3) The third view is that there are in fact two different cellular linings; the first is present in the embryo, undergoes degeneration in the latter part of uterine life and is replaced by a new lining furnished by mesenchyme cells of the lung stroma. According to this theory the air spaces come to be lined secondarily by mesenchymal cells of histiocyte type which produce protoplasmic extensions to cover the alveolar surface while the cell body and nucleus lie in the intercapillary spaces. (Lang/

(Lang, 1925; Rose, 1927; Fried, 1927; Fried & Whitkar, 1927; Fried, 1934; Josselyn, 1935; Loosli, 1935; Geever et al, 1943).

Divergence of opinion between the standard teaching of anatomists and pathologists also exists. The majority of anatomists disagree on the structure of the alveolar wall and question the existence of an alveolar lining. Pathologists, on the other hand, apparently accept the presence normally of alveolar epithelium. which proliferates under various stimuli. (Boyd, 1953; MacCullum, 1940; Bell, 1941; Smith & Gault, 1938; Karsner, 1949; Anderson, 1957; Muir, 1953).

Opinions vary also regarding the alveolar mononuclear phagocytes, and on this question four schools of thought are found. (1) First the alveolar phagocytes (dust cells) originate from the alveolar lining epithelium. (Sewell, 1918-19; Westhues, 1922 & 1925; Cappell, 1923 & 1929; Carleton, 1927; Gross, 1927.) (2) That the phagocytes are mesodermal in origin. (Fried & Whitkar, 1927; Gardner & Smith; Miller, 1932; Fried, 1934; Josselyn, Loosli; Wright, 1935; Unger,Jr., & Wilson, 1935; El Gazayerli; Miller, 1947; Maximow & Bloom; Low & Sampaio). (3) That they arise from the monocytes/

monocytes of blood. (Slavjanski, 1869; Metchnikoff, 1901; Foot, 1927; Low & Deniels, 1952; Low & Sampaio). (4) The fourth, that they arise from the vascular endothelium. (Haythorn, 1913-14; Permer, 1920-21 & 1927; Foot, 1920).

Some of the major contributions may be summarised briefly. They are as follows:

Cappell (1929) worked on the nature of the normal lining of the pulmonary alveoli and the origin of the alveolar phagocytes in the light of vital and supravital staining and made a number of observations from animal experiments. He studied the normal structure of the pulmonary alveoli in mice, guinea-pig and rabbits after intra-vitam staining, with soluble dyes administered by the intravenous, intraperitoneal or subcutaneous routes and observed that the cells lining the pulmonary alveoli showed little or no evidence of vital staining except in conditions of very intense administration. After intense treatment the epithelial lining cells contained a small number of fine dye granules; the histiocytes of the interstitial tissue of the lung on the other hand, became very deeply stained even after small doses of trypan blue and other soluble dyes which were insufficient to produce any/

any vital staining of the alveolar epithelium; the two types of cell could thus be sharply distinguished from one another.

He made the following observations from a number of experiments: (1) After intravenous injection of suspended preparation a proportion of the mononuclear leukocytes of the blood ingested the particulate matter, but no trace of the injected substance was found in the alveolar epithelium or in the histiocytes of the lung stroma. (2) By injecting particulate matter in a fluid medium into the trachea of mice he observed that the particulate matter penetrated into the alveoli and lead to a rapid mobilisation of mononuclear phagocytes which were derived chiefly from the lining epithelium. The flattened nucleated squames and the cuboidal cells (septal cells) both took part in the process. (3) In another experiment in normal mice and in mice previously vitally stained in a variety of ways he observed that after experimentally-produced anthracosis by the inhalation of a sooty atmosphere, soot deposited in the alveoli by inhalation was removed by the action of mononuclear phagocytes, but the cellular response was less rapid than that produced by/

by intratracheal injection of particulate matter in a fluid medium. The appearance of the reacting cells indicated that they were derived from those of alveolar lining. The inhaled soot was subsequently removed chiefly by intracellular transport and was thus passed either into the bronchial lumen to be expelled in the sputum, or was carried into the peribronchial lymphatics to be deposited in the glands of the hilum and mediastinum. (4) He had gathered further evidence as to the origin of the alveolar phagocytes by giving intratracheal injection of carbon suspensions, etc., to animals which have been vitally stained by repeated intraperitoneal or intravenous injection of soluble dye (trypan blue, etc.) Here he found that the alveolar cells occasionally showed much more intense vital staining than was obtained in the normal lung and this was explained by the stimulated condition of the cells by the carbon suspensions.

Cappell (1929) concluded his observations that the normal alveolar lining consists of (a) flattened nucleated squames which form the greater part of the alveolar lining; these cells interdigitate with the following two types of cells; /

cells; (b) large flat non-nucleated squames, which form only a small part of the alveolar lining and appear to be derived from the cells of the first group; (c) the cuboidal septal cells. This is at variance with that currently expressed, according to which the alveolar lining is composed exclusively of non-nucleated squames and the cuboidal septal cells.

Gazayerli investigated the whole problem of cellular lining of pulmonary alveoli by means of animal experiments using different intravital staining methods. (1) In the first series of experiment three rabbits and two guinea-pigs were injected intraperitoneally, daily once, for 11 days with 1 per cent solution of trypan blue in distilled water. The animals were killed on the 12th day and the tissue sections showed that the cells which took up the dye were (a) spindle-shaped histiocytes in the walls of the vessels and bronchi and in the interstitial tissue; (b) the cuboidal cells in the alveolar angles, and (c) the free dust cells. The flattened cells lining the alveoli were unstained. (2) In the second series, a small quantity of carmine or india ink suspended in saline was injected into the trachea of two rabbits/

rabbits and four guinea-pigs subjected previously to intense vital staining as in the first experiment. Animals were killed at various intervals and he found the cuboidal cells swollen with trypan blue granules and some of them contained in addition particles of injected carmine or india ink. A large number of free phagocytes were noted in the alveoli containing both trypan blue and carmine or india ink. The cells lining the alveoli were inactive and remained flattened. From this he concluded that the source of the alveolar phagocytes might be the cuboidal cells which occurred most frequently in the alveolar walls. (3) Next a suspension of saccharated oxide of iron in distilled water was injected intratracheally in five rabbits which were killed at short intervals, ranging from 10 minutes to 2 hours. Here he observed, in the sections (by Prussion blue reaction for iron) a large number of new mononuclear phagocytes in the air sacs and alveoli of the lung, and reported that these cells were obviously derived from the cuboidal cells of alveolar angles as it had been clearly shown that the cuboidal cells became swollen within 10 minutes and their cytoplasm, loaded with iron, presenting an amoeboid/

boid appearance. Very soon these cells were seen separating from the alveolar walls and came to lie free in the lumen of the air cells. They divided by mitosis to provide the alveolar phagocytes. The flattened cells lining the alveoli were totally inactive. (4) To ascertain whether the circulating monocytes participated in the production of alveolar phagocytes, Gazayerli gave intravenous injections of india ink and fine carmine suspensions intratracheally in a rabbit previously intensely stained by intraperitoneal trypan blue. The rabbit was killed after 5 hours. Microscopical examination of the tissue showed that the greater part of the carmine was already intracellular, being contained within large phagocytes many of which showed, in addition, granules of trypan blue. No phagocytes containing ink were observed in the lung alveoli. Apparently no phagocytosis by the capillary endothelial cells had occurred and no blood monocytes containing ink had passed through the capillary walls to gain entrance to the alveoli. Thus the alveolar phagocytes appeared to be derived from the cuboidal cells. (5) He produced active proliferation in the epithelial cells lining the marginal/

ginal alveoli of the lung of rabbits (all vitally stained) by the intrapleural and intratracheal injection of various substances like strontium chloride, etc., to render the flattened cells of the alveolar walls more readily visible fordifferentiation. In all these cases he observed that the marginal alveoli were lined with cuboidal and columnar cells but none possessed any trypan blue or india ink within them, whereas in all cases the septal cells were deeply stained. (6) He also had performed experiments to show the reticulo-endothelial origin of certain cuboidal cells (septal cells) by intratracheal injection of suspension of saccharated oxide of iron in rabbits and subsequently, staining the tissues with silver carbonate (Penfield's modification of del Rio-Hortega method), he showed that like other phagocytes of the reticulo-endothelial system of liver, spleen and brain, etc., the lung also had the silver-stained phagocytes which were conspicuous against grey-coloured septal walls. The alveolar cells were not stained. This showed that the septal cells are part of the reticulo-endothelial system.

From his various observations, Gazayerli concluded that the lung alveoli possess an epithelial/

thelial lining which consists of flat nucleated cells. In addition some cuboidal cells (septal cells) are found which have phagocytic function. These cells are part of reticuloendothelial system and give rise by mitosis to the free alveolar phagocytes.

Thus Gazayerli and Cappell (1929) are complementary in their findings and they both made use of the biological function of cells towards dyes and foreign particles.

Ira Ross and Durham approached the problem experimentally with stimuli more clearly pathological by the study of cellular reactions which resulted from introduction of toxic and non-toxic substances into the lungs of rabbits by the tracheal route. Toxin was made from haemolytic strain of staphylococcus aureus employed by Rigdon and his associates (1934) and was prepared by the method described by Parker, Hopkins and Gunther (1926). The content of one vaccine bottle of toxin was employed throughout all these experiments. Non-toxic substance used was iodised oil of poppy seed, 40 per cent, (Lipiodol Lafay, N.N.R.).

They performed three sets of experiments on rabbits. The first was designed to allow a study/

study of changes in the lung after intratracheal injection of optimal doses of the above substances. Secondly they attempted to determine whether the difference in the results of the toxic and non-toxic substances were qualitative or quantitative by injecting in rabbits intratracheally with varying doses (0.01 ml. and 0.05 ml.) of toxin. The third experiment was to investigate whether or not macrophages of the exudate were capable of wandering from connective tissue into the alveoli and back again. They injected trypan blue intravenously before giving 0.05 ml. of toxin intratracheally in each of three rabbits for 3 days; forty minutes after the toxin was introduced 3 ml. of carmine was put into the trachea of one of the 3 animals. On the day of inoculation another animal received 1 ml. of carmine intravenously followed by 1 ml. by the same route for 2 days. The third animal continued to receive daily intravenous injections of 1 ml. trypan blue. All 3 were killed at 72 hours. Macrophages containing carmine or trypan blue or both were seen in the alveoli and also in the interstitial tissues.

From their observations, Ira Ross and Durham/

Durham claimed to have identified two different types of cells in the alveoli; (1) a non-phagocytic cell lining the alveoli similar and continuous with the epithelial cells of bronchioles; (2) a cell of macrophage type. They claimed that the first type arose from the epithelial cells which lined the alveoli, and the degree of its hyperplastic response was directly proportional to the toxicity of the experimental agent. The macrophage type which responded to both toxic and non-toxic substances was shown to arise from the connective tissue macrophage and to enter the alveoli by free migration across the alveolar epithelial borders.

Our knowledge has been advanced by the use of electron microscopy to study the ultramicroscopic structure of the lung. Workers in this field have agreed that the divergence of opinions regarding the existence of a pulmonary alveolar epithelium in mammals have clearly arisen from the difficulty in demonstrating and identifying such fine structures as the endothelium and epithelium of the alveolar walls with the light microscope. With the higher practical resolving power of electron microscope the fine structure of the pulmonary nucleated alveolar epithelium/

epithelium has been demonstrated as a complete lining facing on the air spaces; thus it appears in health as a true respiratory epithelium. (Low & Daniels, 1952; Low, 1953, 1954; Karrer, 1956; von Breeman & Neustein, 1956).

For electron microscopy, small pieces of lung from normal animal and human lungs (obtained during pneumonectomy) were fixed for 4 hours in 1 per cent osmic acid buffered with sodium barbital to pH 7.4. These tissues were embedded in methacrylate and were cut at 0.06 u. Using similar techniques Low and Deniels, and Low, (1953 and 1954) have noted that the tissues which separate the capillary blood from alveolar air consists of two "cytoplasmic attenuations" which they have named endothelium and epithelium. That basal surface of each is lined with an adherent basement membrane may be potential or real; there is no indication of adhesion between the two basement membranes; when the tissue spaces between them is large, extracellular material and even cells may be interposed as part of the barrier between the blood and the alveolar air.

The developmental origin and phagocytic properties of epithelial lining have been studied/

died by Low and Sampaio by the instillation of thorium oxide (ThO_2) intratracheally in rats. Half-an-hour later it was found that the continuous epithelial lining of the alveoli was not phagocytic in contrast with the macrophages which were free in the alveoli and rested on the attenuated epithelial sheet of the alveolar wall. They noted that the cytoplasm of some epithelial cells closely resembled that of macrophages but they never contained phagocytosed thorium oxide and they rested on a basement membrane rather than on an attenuated epithelial sheet as did the macrophages. They have also observed in some fortuitous fields of lung tissue the situation in respiratory bronchioles where the cytoplasm of the last cuboidal cell attenuated abruptly to a thinness characteristic of alveoli and continued as such over the adjacent capillary.

The cellular lining of the pulmonary alveoli has also been studied extensively in diseased lungs in the human subject. In some pulmonary diseases, air spaces develop which are lined by cuboidal epithelium and have thick connective tissue walls, poor in capillaries. They do not resemble normal alveoli. Groups of alveoli/

alveoli lined by columnar or cuboidal cells are often seen, especially close to a scar or damaged lung tissue, in infarcts, etc., during histological examination of lung at autopsy or removed surgically. The origin of these cells is uncertain. Different opinions prevail as to their origin. Some say (Cooper, 1938; Krahl, 1955) that the cells originate from swelling of the flattened cells which line normal alveoli. Others (Geever et al) believe that 'septal cells' under some stimuli move to form an alveolar lining or to be free in the lumen as phagocytes. The third view (King; Laipply et al, 1955; Spencer & Raeburn, 1956; Maximow & Bloom; Stanton &Stanton & Stouffer, 1957) holds that the lining cells are probably a downgrowth of epithelium from the terminal bronchioles secondary to destruction of the normal process. Evidence to support this view is found in studies of the pathogenesis of certain experimental virus infections in animals and in 'alveolar cell-tumour' in man. (Maximow & Bloom).

Bell (1943) observed that in any disease which brings about marked thickening of the interalveolar septa, with displacement of the capillaries/

capillaries away from the surface and consequent loss of respiratory function, the alveolar epithelium may undergo hyperplasia to form a continuous cubical or columnar epithelial lining; some of these cells may secrete mucin. Almost all the standard text-books of pathology agree with this view.

From the above contributions of different workers on the two-fold problem of (1) the presence of a continuous epithelial lining of the alveolar walls, and its developmental origin, and (2) the nature and origin of alveolar phagocytes, it is now clear from the most thorough and recent experimental and observational work that the presence of an epithelial alveolar lining is recognised. Most of the workers, however, are reluctant to concede phagocytic properties of this epithelial and on theoretical grounds, take the view that intra-alveolar phagocytes must be mesenchymal in origin. But the significance and the recognition of the lined alveoli in diseased lung has still been inconclusive.

ACKNOWLEDGEMENT is gratefully made to Professor G.L. Montgomery of the University of Edinburgh Pathology Department for his considerable guidance in carrying out the research work of my thesis. I am greatly indebted to him for suggesting the subject matter of my work and for his invaluable advice on the production of the thesis:

To Dr. R.F. Ogilvie, Reader in Pathology and Senior Pathologist to the Royal Infirmary of Edinburgh for kindly allowing me to use the autopsy material for my work: to Dr. J. Greening and Dr. D.C. Simpson of the Medical Physics Unit for their kind assistance in carrying out the experiments with radio-active material, and to the staff generally of the Department of Pathology for their friendly co-operation during the period of my attachment to that department as a research worker:

To Mr. T.C. Dodds, Fellow of the Royal Photographic Society, University of Edinburgh Medical Photography Unit, for providing the facilities for the preparation of photomicrographs and in the technicalities of the form of presentation of the thesis and to Mr. J. Paul for taking the photomicrographs. It is a pleasure also to record/

record the assistance I have received from Miss B. Houston and other members of the staff of this department in typing the captions and mounting the illustrations of my thesis.

Technical assistance with the preparation of material has been given by Mr. J. Atherton and Mr. C. Darg. Mr. William Robb provided expert assistance with the care of the experimental animals. To them my thanks are expressed.

Finally, I wish to express my sincere thanks to Mrs. M. Lister, Secretary to the Professor of Pathology, for secretarial help, to Miss P. Watson for typing the thesis, and to other members of the office staff for their co-operation.

PART I

STUDY OF THE REPARATIVE CHANGES OF PULMONARY INFARCTS IN HUMAN AUTOPSY MATERIAL AND IN LUNGS REMOVED SURGICALLY IN TUBERCULOSIS AND BRONCHIECTASIS.

SECTION I : Reparative changes of pulmonary infarcts in human autopsy material.

SECTION II : Reparative changes in lungs removed surgically in tuberculosis and bronchiectasis.

SECTION I

(PART I)

REPARATIVE CHANGES OF PULMONARY INFARCTS IN HUMAN AUTOPSY MATERIAL.

REPARATIVE CHANGES OF PULMONARY INFARCT IN HUMAN AUTOPSY MATERIAL.

INTRODUCTION

Of the non-inflammatory lesions of lung which might conceivably show regenerative changes, infarcts appeared to be worthy of examination. It was realised that the majority of pulmonary infarcts are terminal and that a relatively large number might require to be screened to provide even a few which showed regeneration. Nevertheless, it was appreciated also that even a small number of examples of this degree of organisation would be valuable. So far as it has been possible to ascertain, regeneration of alveoli in relation to pulmonary infarcts has not been recorded. King in his study on "Atypical proliferation of bronchiolar epithelium", interpreted two types of pathogenesis of the proliferating pulmonary epithelium, often found near a zone of relative fixation of lung tissue in atelectasis, fibrosis, infarcts, etc. (1) The first that these proliferating cells may derive from the bronchi and bronchioles, which have ciliated epithelium and a basal layer. The latter proliferates to form compact/

compact mass, which fills up the alveoli and streams from one to another. The cells may be spindle-shaped or with some squamous differentiation. (2) In the second type, there is a proliferating bronchial epithelium, cuboidal or flattened. These cells retain considerable potentialities of growth. They line surfaces primarily, and on occasion proliferate to form a solid mass. Both types of growth may coexist. He, however, did not mention specifically pulmonary alveolar regeneration. Stanton and Stouffer found similar bronchiolar and alveolar epithelial hyperplasia in and around the healed experimental pulmonary infarcts in rabbits. Discussing the character of the hyperplastic epithelial cells in the alveoli they commented that "whether this hyperplastic response is an abortive attempt at regeneration is a moot point, but we are impressed by the fact that mitotic activity continued in these hyperplastic cells for as long as four months after the original injury."

In this section, attempts have been made to study the cytological relationship of repair of pulmonary infarcts with that of experimental lesions in animals.

MATERIAL/

MATERIAL AND METHOD

Material collected for the study of organisation of infarcts were the post-mortem records, histological slides and paraffin blocks of cases of pulmonary infarcts of the Royal Infirmary of Edinburgh. Fresh sections were prepared for microscopical examination from the paraffin blocks of lung tissue containing areas of infarction. Thick and thin sections were stained with haemotoxylin and eosin, modified picro-Mallory method for elastic tissue and, for iron-pigments, the prussian blue reaction.

Out of total number of 16137 autopsies performed in the Royal Infirmary during the period of 25 years, from 1932 to 1956 inclusive, 851 cases (5.27 per cent.) were found to have definite infarcted areas in one or both lungs. Suggestive clinical findings of pulmonary infarct had been noted in many cases during their illness and were helpful in estimating the possible date of the lesion in the lung. In this series of 851 cases of pulmonary infarcts, 30 instances were found which revealed organisation and proliferation of epithelium in and around the infarcted zone; 11 of them have been discussed here as a representative group.

From/

From the autopsy records the following general information was obtained:

Of the 851 cases, 54 per cent had infarcts in both lungs, 32 per cent in the right, and 13 per cent in the left lung alone. The basal part of the right lung was involved in 70 per cent cases. Age incidence varied from as low as 13 years (a boy) and 16 years (a girl) to as high as 98 years (male) and 91 years (female) of age. The greater frequency was found to be between 45 to 65 years. The lower-age group was usually found to have had cardiac rheumatic disease from childhood, some congenital anomalies either of the heart or the lungs or both, or they might be victims of physical injuries (fracture, surgical operations, etc.) The older group of cases with pulmonary infarction had suffered usually from long-standing heart diseases with passive venous congestion of various organs. Post-injury and post-operative cases were exceptions. In about 60.1 per cent of the total cases there was passive venous congestion with or without gross cardiac pathology. Hypertensive heart disease was found in 175 cases out of total 510 cardiopathies; coronary thrombosis without definite cardiac infarction or/

or fibrosis numbered 11C, cardiac infarction and/or fibrosis accounted for the remainder.

REPORTS OF CASES

Case 1.

Female, 71 years, had suffered from breathlessness on exertion for a number of years. For 5 weeks she had been breathless at rest and for 2 weeks she had palpitation, and oedema of both ankles and feet. There was a history of articular rheumatism between the ages of 16 and 30 years. On examination: auricular fibrillation, blood pressure 180/70mm. Hg., sacral and ankle oedema, congestion of neck veins, enlarged liver, aortic incompetence and mitral stenosis. Died suddenly from right heart failure.

Relevant Autopsy Summary: Chronic rheumatic carditis, mitral stenosis, right ventricular hypertrophy and dilatation, generalised chronic venous congestion. Lung: Organised infarct and brown induration in the left lung.

Microscopical Examination: The central part of the lung lesion is a well-established haemorrhagic infarct; the red blood corpuscles which pack the alveoli cannot always be identified/

fied as discrete structures. The alveolar walls have lost their nuclear staining; some of them have ruptured to form emphysematous bullae. In the margins of the infarct there is evidence of bronchial regeneration. Fig. 1 shows cubical epithelium replacing ciliated respiratory epithelium in a small bronchus; there are clumps of dark staining epithelial cells in the lung tissue above. In Fig. 2 there is another small bronchus with several bronchial buds lined by cubical epithelium beside it, one of them opening from the lumen. No elastic tissue can be seen in relation to these structures. An artery shows endarteritis. Fig. 3 is from the contiguous lung. There are many alveolar spaces containing phagocytes, lined by dark cubical cells and separated by collapsed lung.

The infarct is probably not more than 5 weeks old.

Case 2.

Male, 81 years, admitted with a long history of heart trouble and angina pectoris. 9 weeks prior to admission arterial thrombi and embolus of femoral artery were diagnosed. Subsequent gangrene of right leg with cardiac fibrillation/

fibrillation. Amputation of right leg 2 days after admission. For the last two weeks frequent episodes of sudden dyspnoea of short duration. Died suddenly 22 days after his admission in the hospital.

Relevant Autopsy Summary: Coronary occlusion, myocardial infarct, cardiac hypertrophy and dilatation, cystic kidneys, prostatic cancer. Lungs: Hypertrophic vesicular emphysema of upper lobes, infarcts at both bases.

Microscopical Examination: Lung sections show small metastases of prostatic carcinoma. The infarct available for examination was related to an embolus in a branch of the pulmonary artery. Figs. 4 and 5 are examples of widespread bronchial budding in the periphery of the infarct. (The dark area in the upper part of Fig. 4 represents the haemorrhagic zone). In Fig. 4 also there is the interesting feature of organisation and recanalisation of the obstructed pulmonary artery. Fig. 6 shows the cubical cells in the alveoli of adjacent lung.

The estimated age of this lesion is probably greater than the date of the amputation which took place 3 weeks previously.

Case 3.

Adult/

Adult male, coronary thrombosis 1953; anginal symptoms since. Admitted June, 1954, with cardiac failure. Admitted again October, 1955, with pulmonary infarct and congestive failure; re-admitted on 7.11.55 with severe congestive failure. Became severely dyspnoeic, confused and restless; coma and death 2 days after admission.

Relevant Autopsy Summary: Myocardial infarct; congestive cardiac failure. Congested oedematous lungs with old and recent infarcts.

Microscopical Examination: Sections from the centre of the pulmonary infarcts show necrotic alveolar walls with some disorganisation of the elastica (Fig. 7). Around the more recent infarcts the alveolar walls are congested and the alveoli contain oedema fluid, red blood corpuscles and histiocytes full of haemosiderin. Organisation has started from the periphery of the infarct and fully developed fibroblasts cells and capillary buds are seen to penetrate from the edge of the old infarct (Fig. 10). A viable bronchus at the periphery has a characteristic appearance (Figs. 8 & 9). Its respiratory cells are replaced by cubical cells, alveoli on its periphery are partly re-formed/

formed by a process of organisation and many darkly-staining bronchial buds can be seen. Several thrombosed blood vessels are present and in one of them, there is an organised, canalised thrombus (Fig. 10).

Case 4.

Female, 68 years, had suffered from severe dyspnoea for 3 months with loss of weight and clubbing of fingers. Her sputum contained tubercle bacilli and her chest X-ray showed a tuberculous bronchopneumonia. She was cyanosed. Blood pressure normal. After 14 days in the hospital she collapsed suddenly and died.

Relevant Autopsy Summary: Limited tuberculosis at the hilum of the right lung, pulmonary endarteritis and cor pulmonale; congestive cardiac failure, adenoma of the adrenal cortex and organised pulmonary infarcts.

Microscopical Examination: Sections of the lung confirmed tuberculous lesion of the lungs and also non-specific fibrosis. In the fibrotic areas (Fig. 11) newly-formed bronchi and alveoli are lined by hyperplastic cubical cells. Bronchial buds are seen sprouting out. (Figs. 11, 12 & 13). The nature of hyperchromatic/

hyperchromatic cells is well shown in the high power photomicrograph (Fig. 13) which shows also their continuity with the flattened elongated cells in the dilated bronchus.

Case 5.

Female, 61 years, with severe attacks of breathlessness for 3 months before admission on 4.1.50; died on 6.2.50. She had cough with blood-stained sputum, was cyanosed, pyrexial with peculiar muddy complexion. There was widespread crepitations specially on the right base where percussion node was dull. She died suddenly.

Relevant Autopsy Summary: Massive recent infarction of most of right middle lobe. The infarcted region was dark purple in colour owing to haemorrhagic consolidation and it was bounded by a narrow granulating zone; numerous small wedge-shaped infarcts of different ages throughout the middle and lower lobes; brown induration and chronic venous congestion of lung; hypertensive myocardial hypertrophy; coronary occlusion; diffuse dilatation of the heart; nephrosclerosis; phlebo thrombosis of veins of left leg. Age of infarcts uncertain/

tain, but probably about 3 months in case of older lesions.

Microscopical Examination: Sections from different areas of the lungs revealed chronic venous congestion with numerous heart failure cells. Infarcts of two ages were present. In the more recent ones the pulmonary arterial branches contained thrombus which possessed the characteristic laminated structure associated with ante-mortem formation, and the parenchyma was necrotic. Abundant well-preserved red blood corpuscles and polymorphs were seen in the alveoli. In the older infarcts the arterial thrombi had undergone organisation by ingrowth of fibroblasts and capillaries from the intima; recanalisation had taken place in some branches. In the periphery of the infarcts organisation was quite advanced. A particular area of the infarct showed a large bronchus with squamous epithelium. (Fig. 14). In another bronchus, the hyperplastic dark-staining type of cubical epithelium formed bronchial buds directed towards the infarcted area. Some areas of the infarct (Fig. 15) had epithelial clumps with dark-staining cells where scanty mitotic figures were found in the epithelial cells. Extensive

foetalisation of lung tissue was found in another part of an organised infarct where formation of bronchial buds were quite evident. Dark-staining, atypical, newly-formed alveoli from the bronchial buds were seen lined by cubical to flattened cells. (Fig. 16). In some of the bronchial buds there was fresh blood in the lumen.

Case 6.

Female, 66 years, with a history of heart failure for 3 years. Pain in right side of chest for 1/52 and vomiting one day before admission. On examination, crepitations at both bases of the lungs, breath sound diminished and dullness at right base, oedema of sacral region and both legs. Chest X-ray showed cardiac enlargement and bilateral congestion of the lungs. She died of heart failure 14 days after admission in the hospital.

Relevant Autopsy Summary: Congestion of both lungs, a few emphysematous bullae at the anterior margins, infarcts in the right lower lobe.

Microscopical Examination: Sections from the infarcted areas revealed old organised infarct, partly fibrosed, Epithelial cell proliferation/

proliferation had occurred in the fibrous zone and their penetration takes the shape of bronchial buds, (Fig. 17 - top left), and abundant small capillaries. In some areas regenerated alveoli with cubical cell lining are present with alveolar phagocytes, (Fig. 18), containing the necrotic debris.

Case 7.

Male, 50 years, suddenly became dyspnoeic one night about 7 weeks prior to admission. Since then he had to sleep sitting up; he was febrile. On admission (27.11.49), there was oedema of ankles and sacrum, followed by a pleural effusion on the left side. Thoracocentesis performed on 13.1.50. He became more dyspnoeic and died on 23.1.50. Blood pressure was 170/128mm. of mercury while in the hospital.

Relevant Autopsy Summary: Pulmonary embolism. Considerable atheroma in the pulmonary artery, old and recent infarcts in the lungs.

Microscopical Examination: Sections from the zone of infarcts showed advanced organisation with foetalisation of lung tissue in the sub-pleural region extending into the area/

area of infarct. (Fig. 20). The original structure of the lung was only barely discernable amongst eosinophilic homogeneous zones. Many related pulmonary arteries were occluded by thrombus and some of these thrombi had been canalised. (Fig. 19). Dark-staining epithelial cell proliferation in clumps was abundant in the areas next to sub-pleural zone. New capillary formation and fibroblastic reaction were seen at the periphery of the infarct.

Case 8.

Female, 40 years, had attacks of breathlessness and tiredness for 5 months, with subsequent chronic cough, tightness of chest and headache. History of bronchiectasis and tuberculosis at 41 years of age. Pleurisy 12 years ago. On examination she was cyanosed and breathless; had tachycardia and had lost a great deal of weight. Died suddenly 4 weeks after her admission in the hospital.

Relevant Autopsy Summary: Small bronchiectatic dilatations in upper lobes of both lungs; wedge-shaped areas of organised infarcts in the lower lobes.

Microscopical Examination: Sections from the infarcted area reveal no sign of alveolar walls/

walls. Elastica is quite irregular but in great abundance in the organised part. The whole area of the infarct is fully organised and most of the organised part is fibrosed. In parts young capillaries and fibroblasts are seen proliferating, (Fig. 21). Regenerated alveoli lined by cubical cells can be seen in the periphery of the infarct.

Case 9.

Male, 40 years, complained of left-sided chest pain 15 days before admission in the hospital. Two days later he had haemoptysis and blood-stained sputum persisted. On the day before his death he developed acute right-sided pain in the chest and was admitted to hospital with 99°F temperature. Pulse 120 per minute and respiration 55. He improved for a little while but died within 20 minutes being seized by an acute right-sided chest pain. X-ray showed right ventricular hypertrophy.

Relevant Autopsy Summary: Large infarct in the left lower lobe, smaller infarct in the right lower lobe. Hypertrophy and dilatation of the right side of the heart. Moderate generalised atherosclerosis.

Microscopical Examination: Sections of lung from the infarcted areas show all grades of/

of organisation. In some areas the alveolar pattern is replaced by new capillary blood vessels and fibroblasts, in other parts the bronchial proliferation is specially marked, (Figs. 22, 23 & 24) with many dark-staining epithelial cells sprouting from the bronchial epithelium. There are several thrombosed vessels.

Case 10.

Female, 63 years, had a 20 years history of ankle oedema, cyanosis and dyspnoea on exertion. Admitted (15.2.56) with right basal pneumonia, tightness in the chest and haemoptysis. She had had haemoptysis on two occasions about a year ago. Suddenly collapsed and died (18.2.56). One week before death she had suffered from paroxysmal tachycardia followed by haemoptysis and signs of cardiac failure.

Relevant Autopsy Summary: Both lungs - congested and oedematous; haemorrhagic infarcts at both bases; partially fibrosed infarct in right middle lobe. Mitral-valve incompetence, post-rheumatic mitral stenosis, hypertrophy and dilatation of right heart, chronic venous congestion of liver, spleen and kidneys. The woman/

woman seemed to have repeated attacks of pulmonary infarct during illness, the oldest one seemed about one year old.

Microscopical Examination: (Figs. 25 - 29). In the older, organised areas of pulmonary infarcts there are relatively widespread spaces lined by cubical and flattened cells which replace almost the whole of the organised infarct. Clumps of regenerating epithelial cells are to be seen and the active growth of capillary blood vessels is a feature.

Case 11.

Male, 61 years, had abdominal pain and dyspepsia, general malaise and fever for about 6 weeks. A month ago he had sudden pain in right lower chest, cough and purulent sputum. On the day before admission the left leg suddenly became swollen and tense. On examination he was tired, toxic-looking, perspiring and in poor general condition. His condition deteriorated and died in cyanosis.

Relevant Autopsy Summary: Carcinoma body of pancreas with metastasis to liver and regional lymph nodes: thrombosis of widespread distribution affecting pulmonary arteries, vessels in kidneys, femoral, external and common iliac veins/

veins and inferior vena-cava and resulting pulmonary and renal infarcts.

Microscopical Examination: The tissue has been taken from pneumonic and infarcted areas. Sections from the infarct show a usual appearance. New air spaces are in process of formation, mainly by outgrowth of bronchial buds from a pre-existing bronchus. Plump, well-stained cells line these spaces and the remarkable feature is that the cells are actively phagocytic and have engulfed red blood corpuscles from vascular, haemorrhagic region of the organising infarct. (Figs. 30 - 33).

DISCUSSION

From the examples cited in this section, it is quite clear that infarcts of lung in the adult are capable of organisation with the formation of air spaces. This type of reaction probably occurs relatively seldom because the majority of patients develop infarcts as terminal events. But the infrequency of this reparative change does not diminish its importance as indicating first that an infarct is a stimulus in this respect similar to trauma and secondly, that regeneration of lung alveoli is/

is within the capacity even of engorged congested lungs of adults.

The re-formation of the lung tissue in an infarcted area occurs in two ways, at the alveolar and bronchial levels. Reformation of alveolar spaces is shown often by the appearance of cubical cells lining the alveolus in whole or in part; this is the type of change seen frequently in the re-opening of existing alveoli in inflammatory lesions, the so-called unresolved pneumonia: such a relation is to be noted with interest but does not constitute true regeneration. The latter is shown by the appearance of air spaces, sometimes slit-like and tortuous in the connective tissue around an infarct and by the fact that such spaces acquire lining cells. In infarcts, as in wounds in lung, there is no doubt that this occurs and usually, there is clear evidence that the lining cells are extensions of bronchial epithelium. Figs. 4, 8, 12 & 13 may be mentioned as illustrative examples though the sections contain many other instances. Proliferative change is to be seen in the lining cells of parent bronchi, the tall columnar cells giving way to flattened cells of endothelial/

thelial pattern: metaplasia to squamous or stratified cells also takes place in some cases.

As is so often the case where records have to be searched, it has proved impossible to estimate more than roughly the age of the infarcts. It is clear, however, that for organisation to proceed to the stage of re-formation of alveoli and establishment of bronchial buds, several weeks must elapse. There is no reason why the re-organisation should not proceed to complete repair though no example of this has been seen. The most interesting case is number 11, where the proliferating epithelium in situ in the bronchial buds has become phagocytic of red blood cor-:puscles: the significance of this point will be discussed later.

SUMMARY

Eleven representative cases of organised pulmonary infarcts were studied from a series of autopsy cases.

Microscopical sections of the organised areas of these lesions reveal definite evidence of regeneration of pulmonary tissue by formation/

formation of bronchial buds, new bronchi and alveoli from the viable bronchi and bronchioles in and around the affected zone.

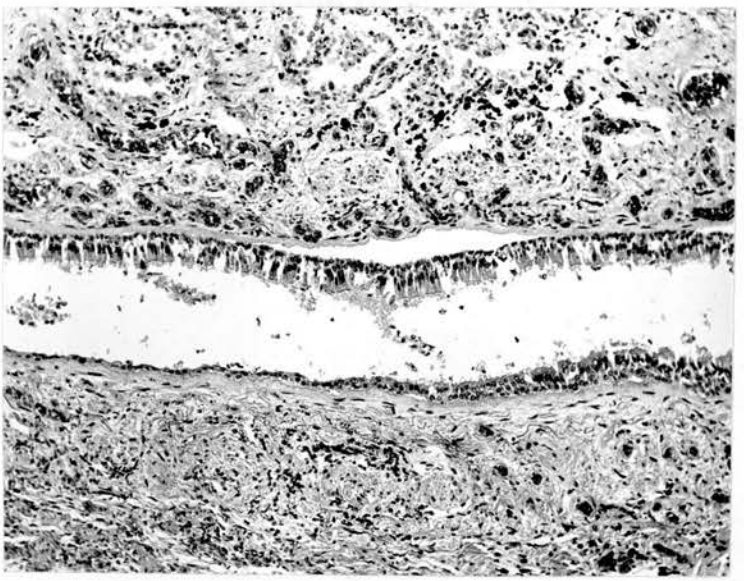

Fig.1,(Case 1.) : 5 weeks old organised infarct of human lung showing transformation of ciliated epithelium into cubical and flattened cells of a small bronchus. x 100.

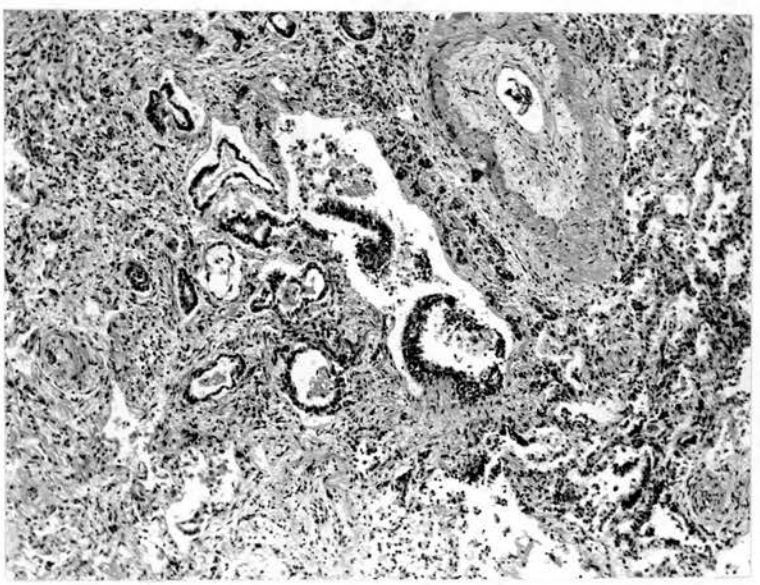

Fig.2,(Case 1) : Organised infarct of human lung. Epithelial bud formation in the organised area and an artery showing endarteritis. x 70.

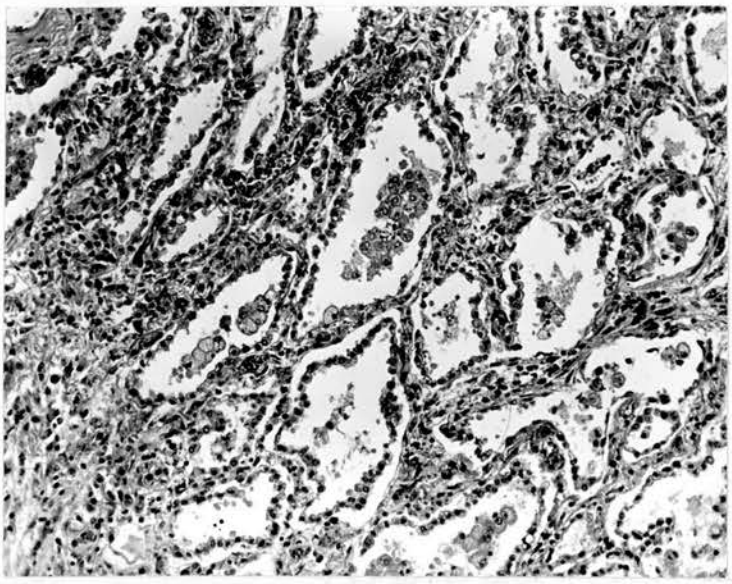

Fig.3,(Case 1.) : Same case as above showing cuboidal cell lining of alveoli near an organised infarct with phagocytes in their spaces. x 135.

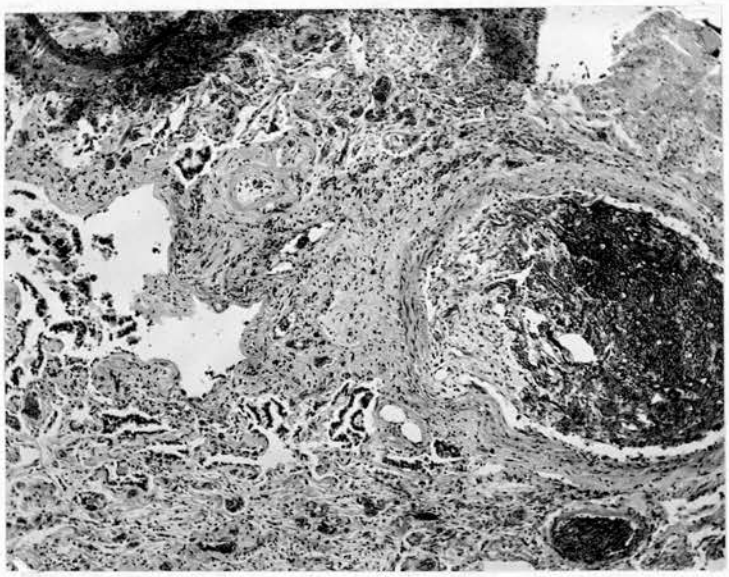

Fig.4,(Case 2.) : Organised infarct of human lung showing bronchial budding, organisation and recanalisation of an obstructed pulmonary artery. Dark area in the upper part represents haemorrhagic zone. x 65.

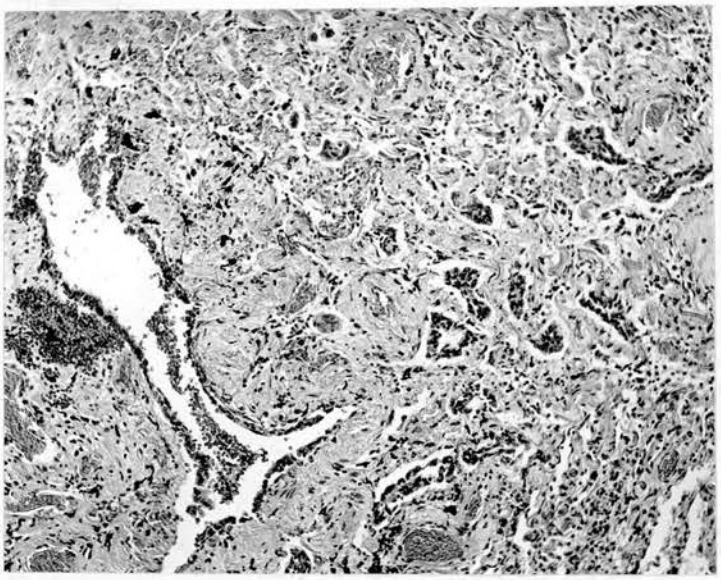

Fig.5,(Case 2.) : Organised infarct of human lung showing wide-spread bronchial budding in the peripheral zone. x 95.

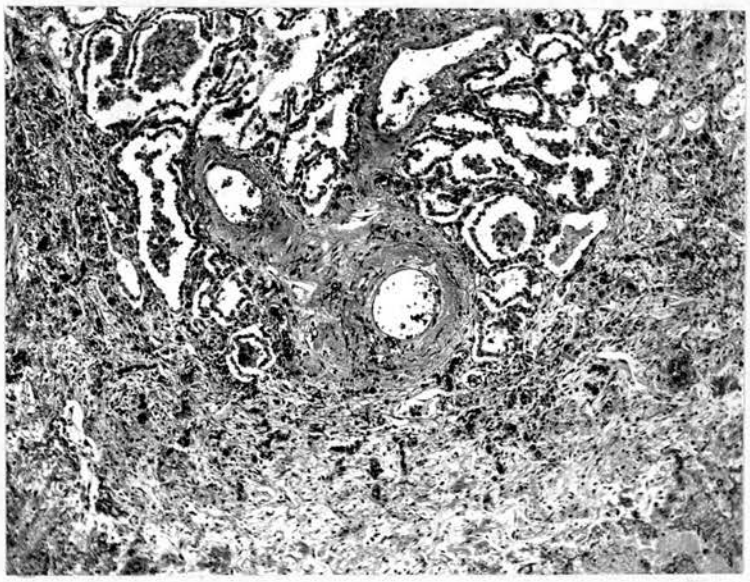

Fig.6,(Case 2.) : Same case as Fig.5 showing alveolar walls lined by cubical cells. Dark masses of epithelial cell proliferation in the fibrous zone. x 70.

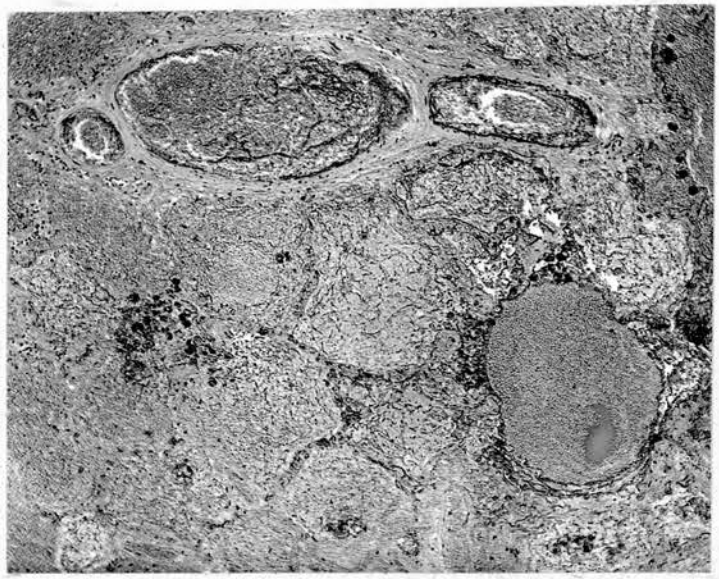

Fig.7,(Case 3.) : Pulmonary infarct showing necrotic alveolar walls wwith some disorganisation of elastica. x 80.

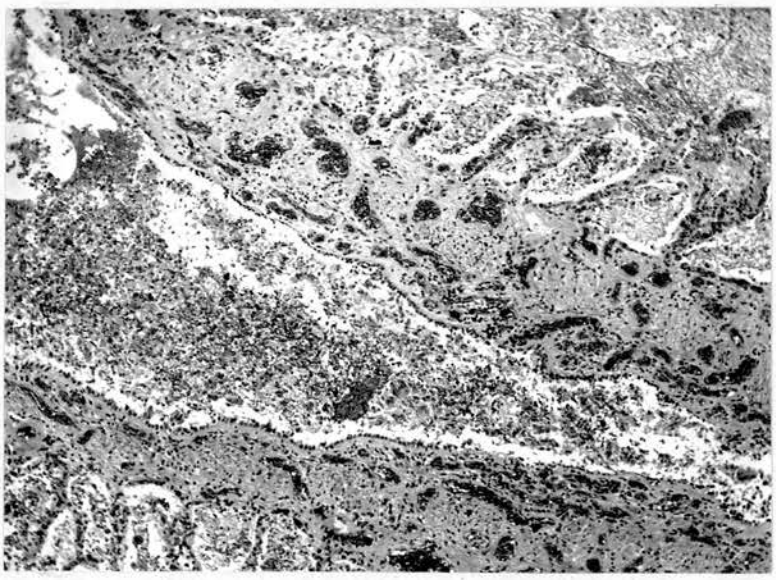

Fig.8,(Case 3.) : Peribronchial organisation with bronchial buds and new alveoli formation in an area of old infarct of human lung. The bronchus is lined by cubical, and in some parts by flattened cells. x 70.

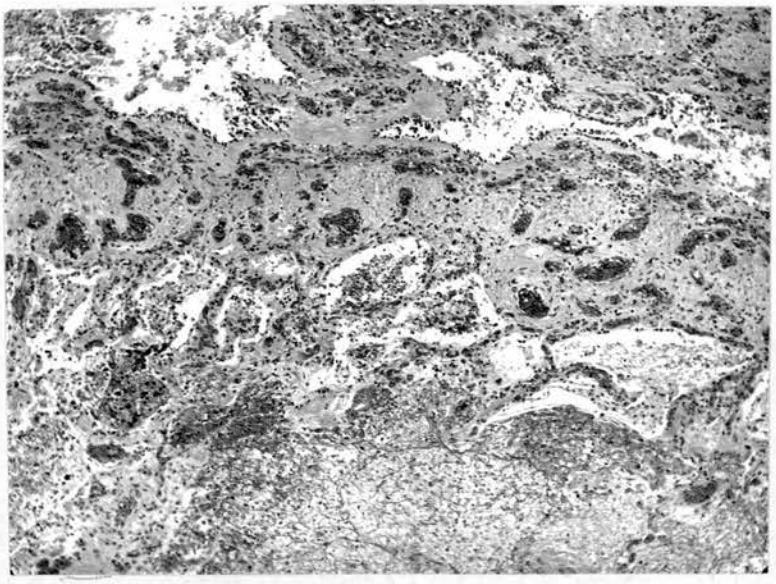

Fig.9,(Case 3.) : Continuation of the above figure from right end of the bronchus. x 70.

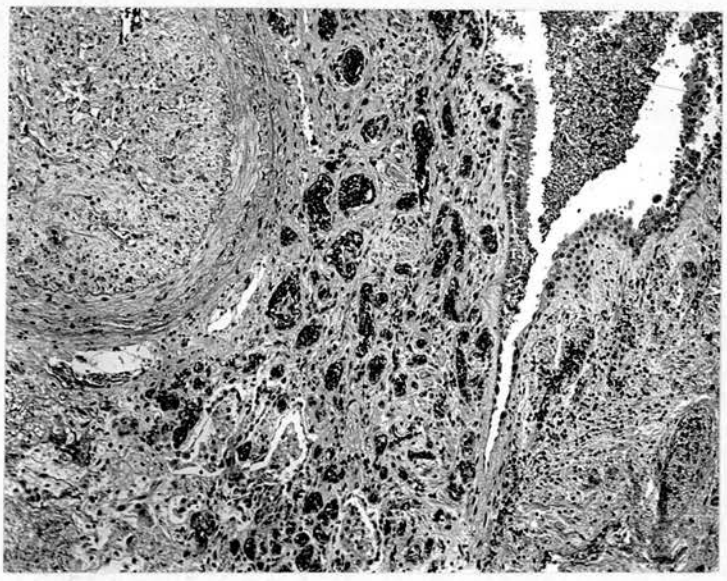

Fig.10,(Case 3.) : Organised infarct of human lung showing an organised thrombus of a blood vessel at the periphery and bronchiolar buds penetrating into the organised area. x 95.

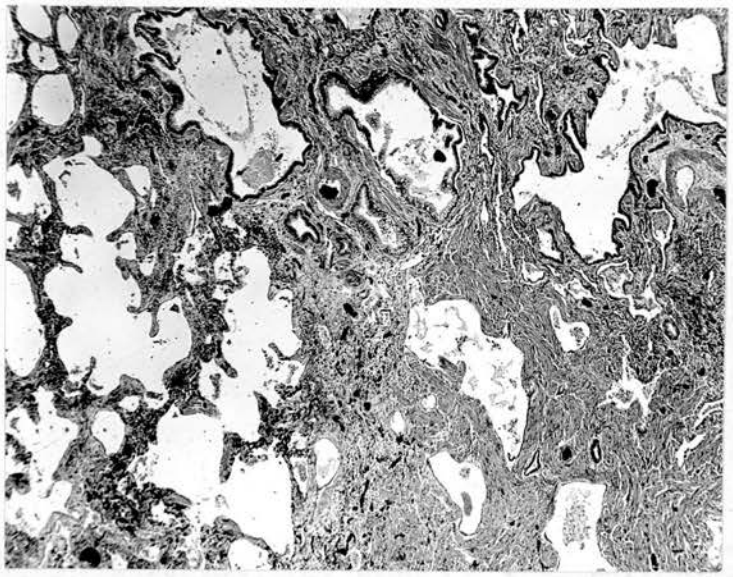

Fig.11,(Case 4.) : Newly formed bronchi and alveoli are seen lined by hyperplastic cubical cells in an organised infarct of human lung. x 30.

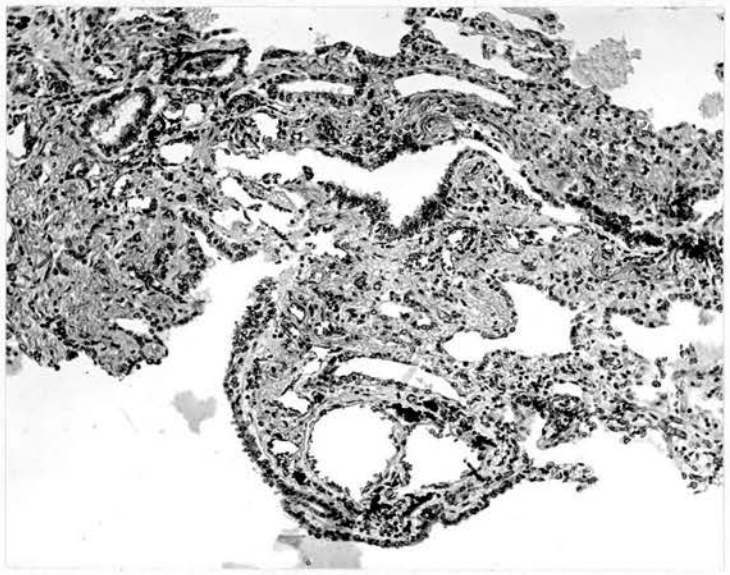

Fig.12,(Case 4.) : Peripheral part of an organised pulmonary infarct showing new bronchi and alveoli lined by cubical cells. x 120.

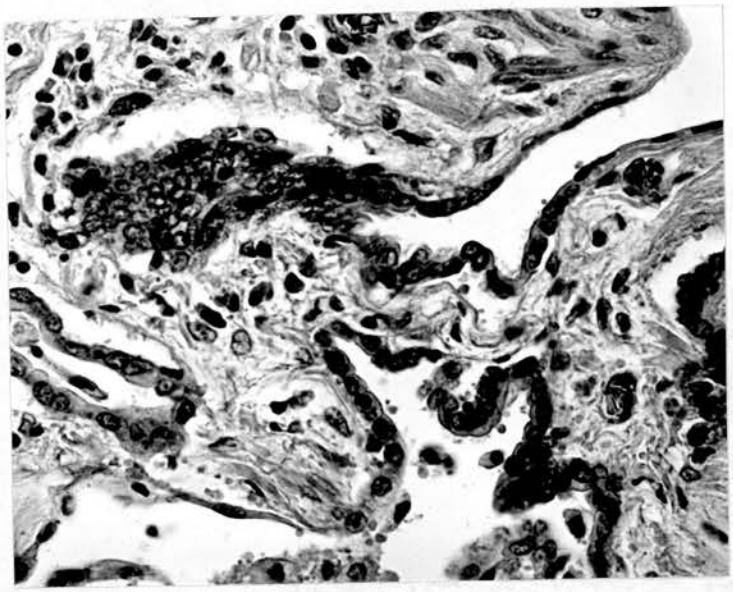

Fig.13,(Case 4.) : High power of Fig.12, showing regeneration of bronchiolar epithelium growing out into alveoli. The cubical cells show their contiguity into flattened elongated cells. x 400.

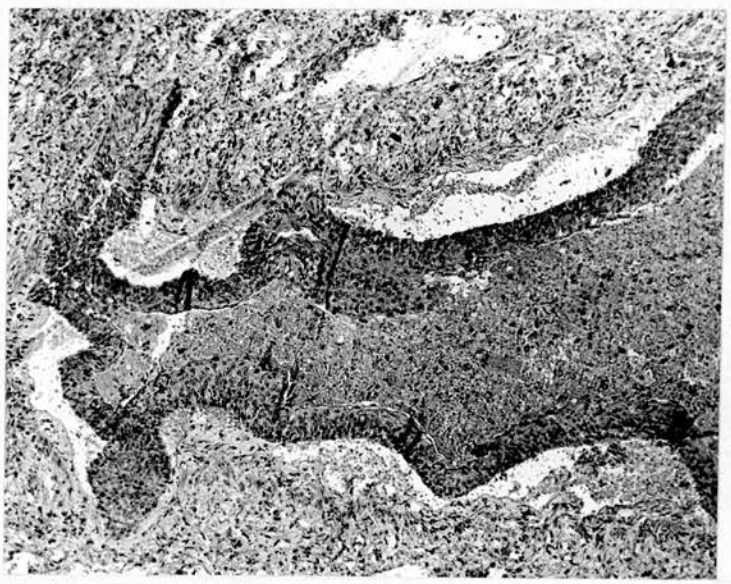

Fig.14,(Case 5.) : Squamous metaplasia of bronchial epithelium in a 3 month old organised infarct of human lung. x 80.

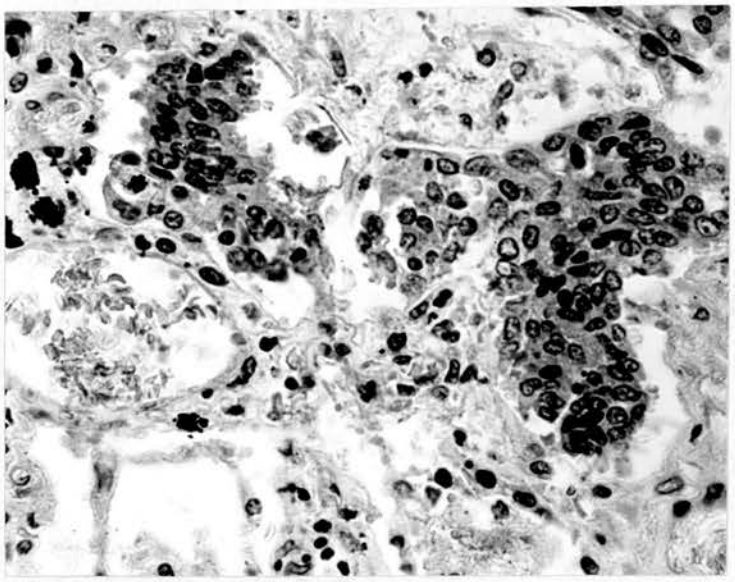

Fig.15,(Case 5.) : Early (left) and late (centre) phase of mitoses are seen in the epithelial clumps forming bronchial buds directed towards the organised infarct of human lung. x 400.

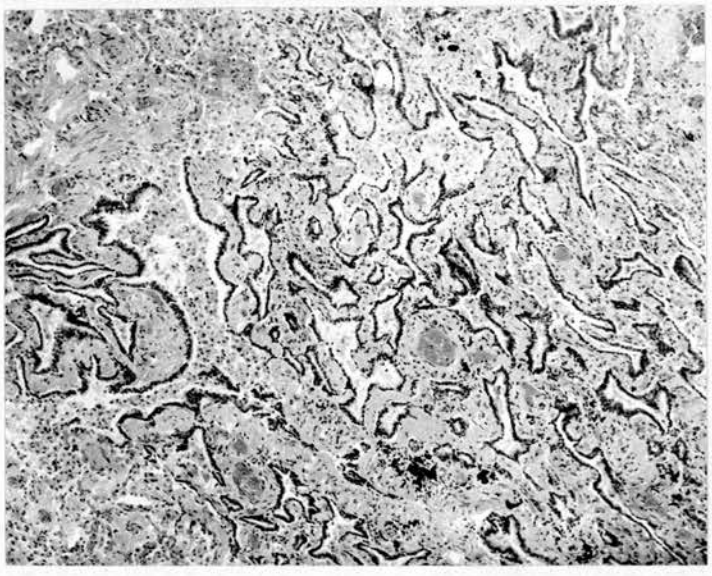

Fig.16,(Case 5.) : 3 months old infarct of human lung showing newly formed bronchi and a typical alveoli lined by cuboidal cells. x 80.

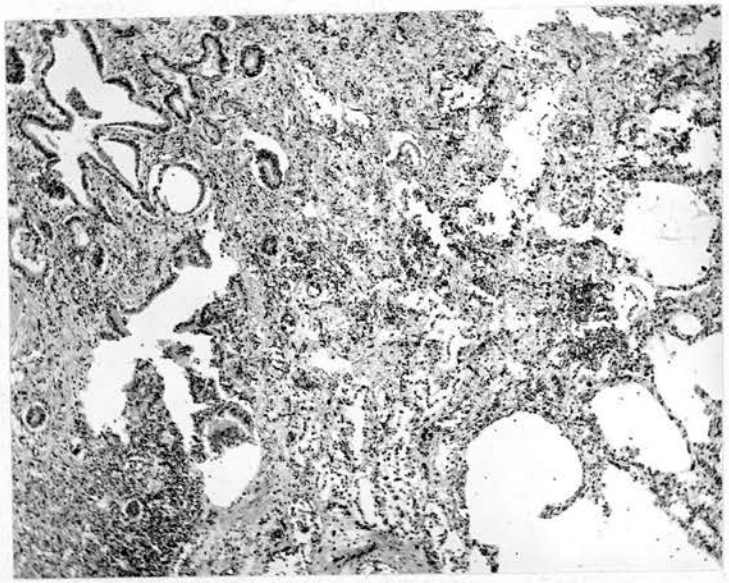

Fig.17,(Case 6.) : Infarct of human lung showing bronchiolar and alveolar regeneration. x 65.

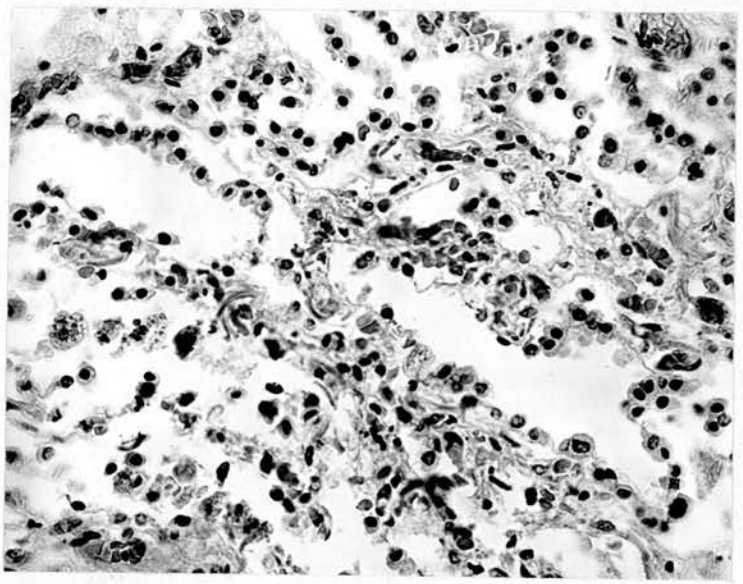

Fig.18,(Case 6.) : Regenerated alveoli with cubical cell-lining are seen in an organised infarct of human lung. Alveolar phagocytes containing necrotic debris are also seen in the area. x 325.

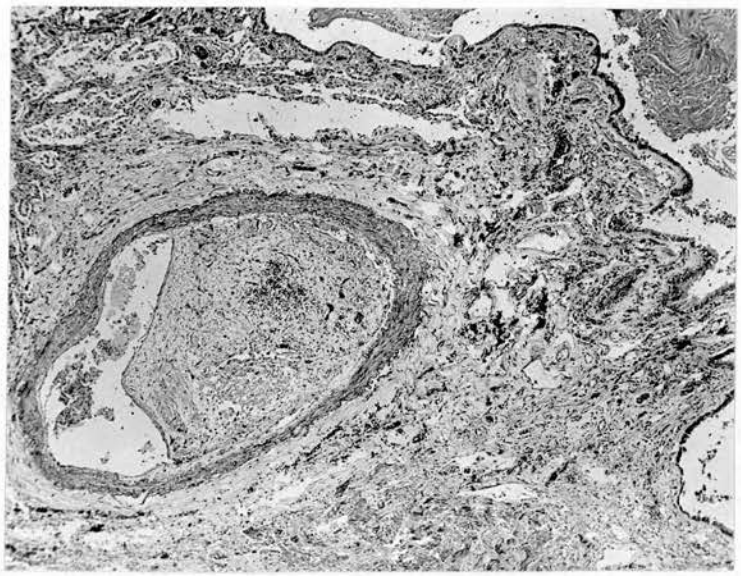

Fig.19.(Case 7.) : Left : a recanalised thrombosed vessel at the periphery of an organised infarct ; right : bronchiolar budding and epithelial metaplasia. x 60.

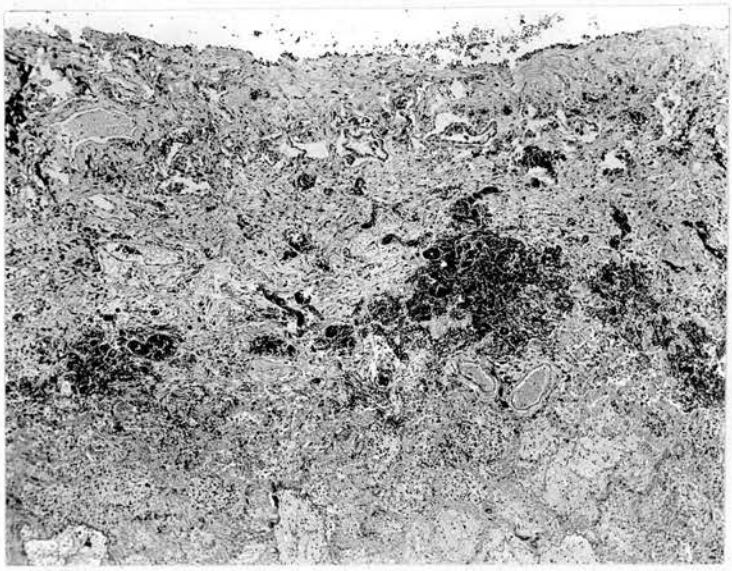

Fig.20.(Case 7.) : Organised infarct of human lung showing subpleural foetalisation of lung tissue. x 60.

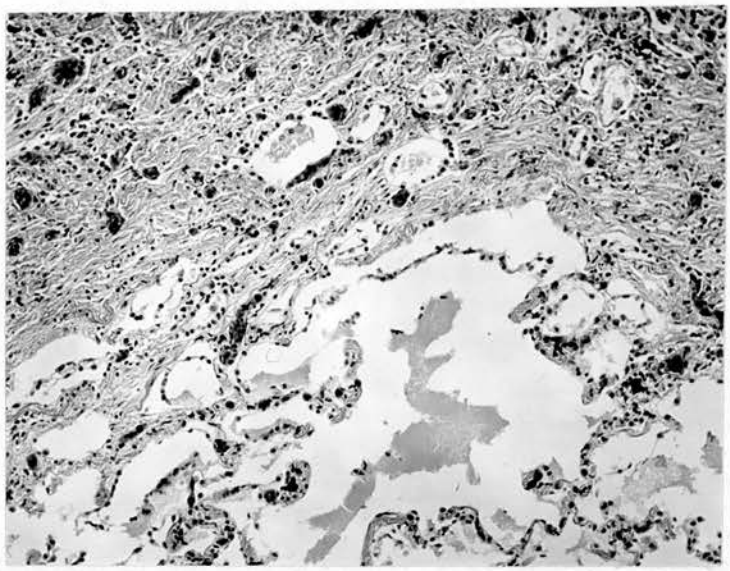

Fig.21,(Case 8.) : Old human pulmonary infarct showing young capillaries and fibroblasts and some regenerated alveoli lined by cubical cells. x 120.

Fig.22,(Case 9.) : Organised infarct of human lung. Some bronchial budding and new alveolar spaces in relation to large bronchi. x 60.

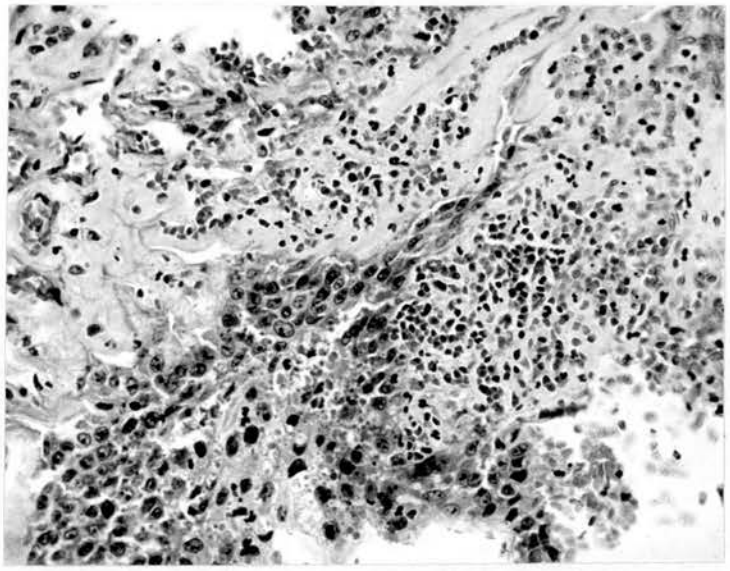

Fig.23.(Case 9.) : Regenerating bronchial epithelium in an organised infarct of human lung. x 260.

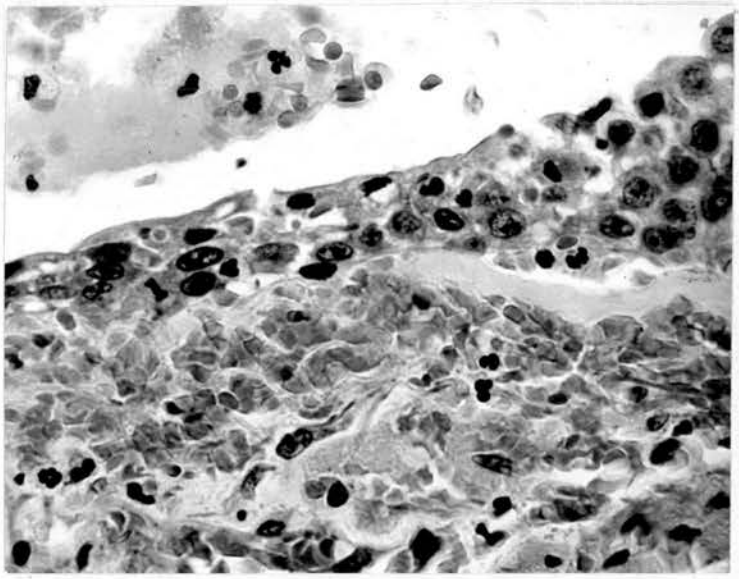

Fig.24.(Case 9.) : Mitotic activity can be seen in this higher power view of the above figure (23). x 550.

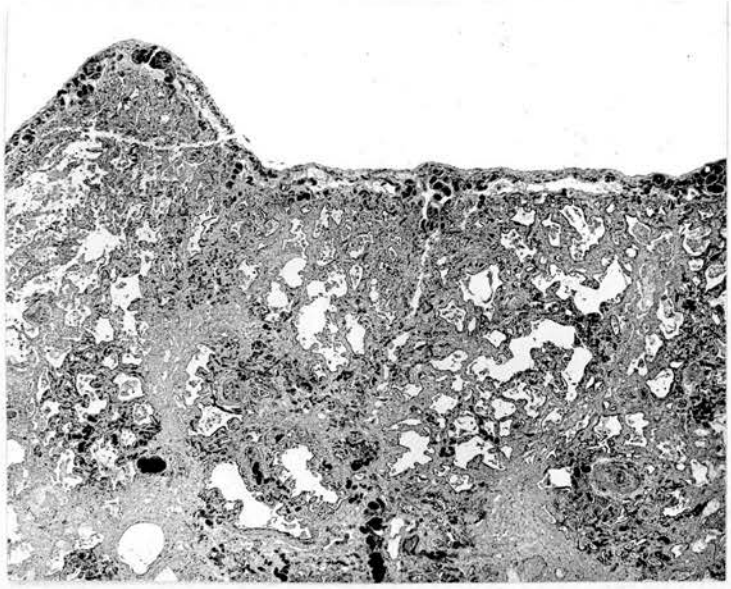

Fig.25,(Case 10.) : Old infarct of human lung. Almost the whole area is re-aerated by newly-formed bronchi and alveoli. x 10.

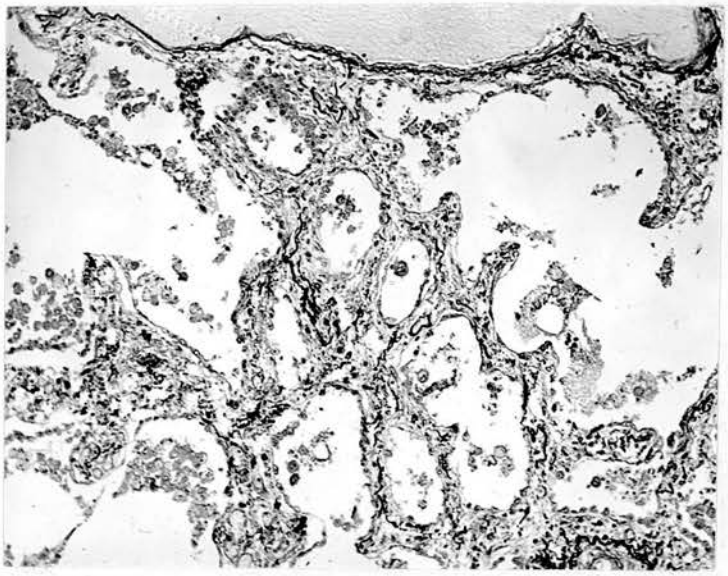

Fig.26,(Case 10.) : New, dilated alveolar spaces in the periphery of an organised infarct of human lung. x 95. (Weigert.)

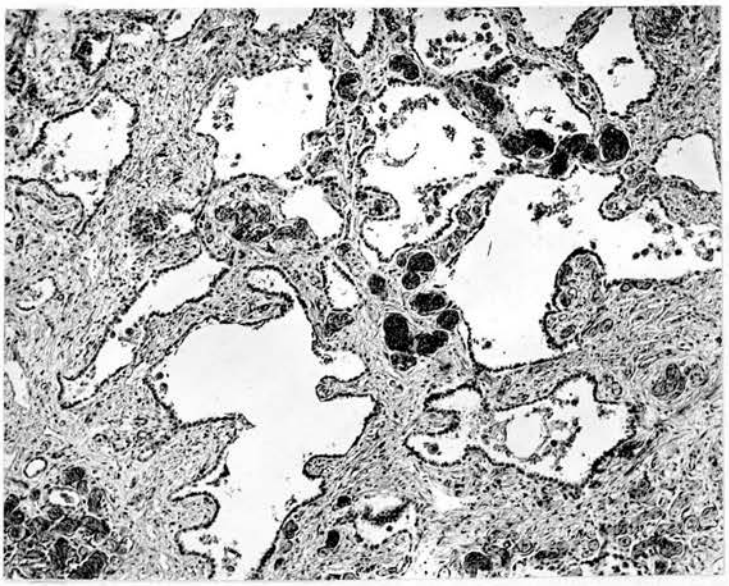

Fig.27,(Case 10.) : High power view of Fig.25, to show the newly formed alveoli. x 60.

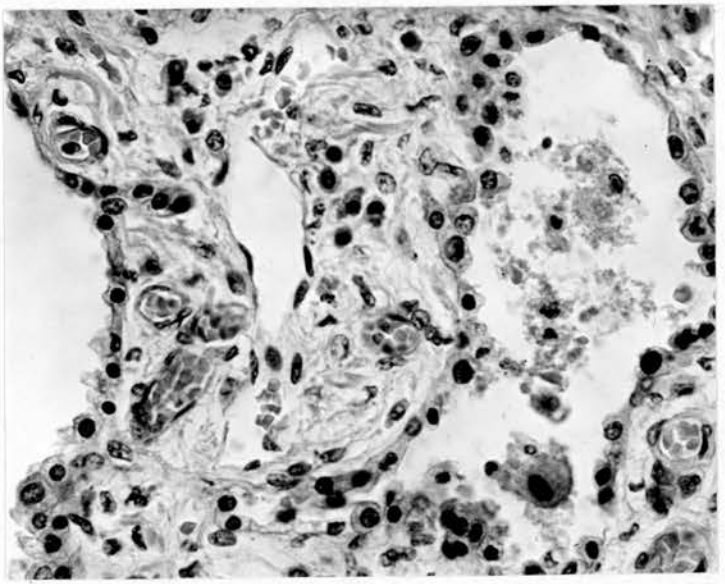

Fig.28,(Case 10.) : Higher power view of Fig.25, to show cellular lining of the regenerated alveoli. x 375.

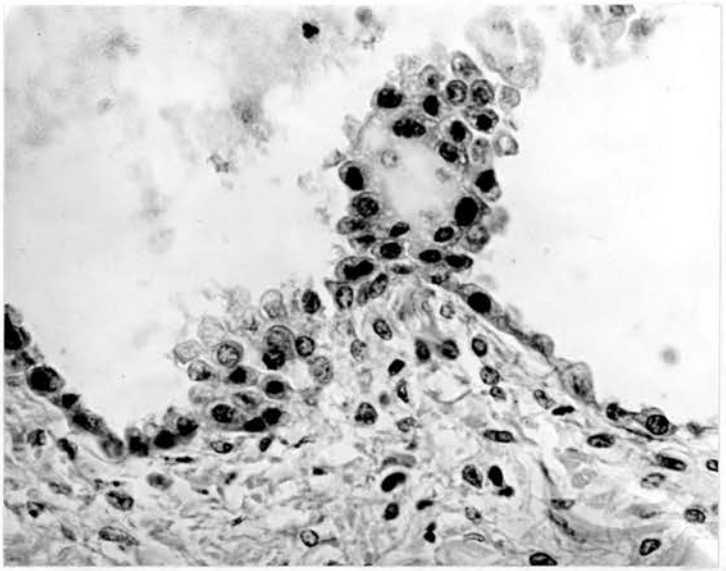

Fig.29,(Case 10.) : Some mitoses are seen in the alveolar epithelium formed from bronchiolar bud. x 375.

Fig.30,(Case 11.) : Organised infarct of human lung showing bronchial buds lined by well-stained cubical cells. x 70.

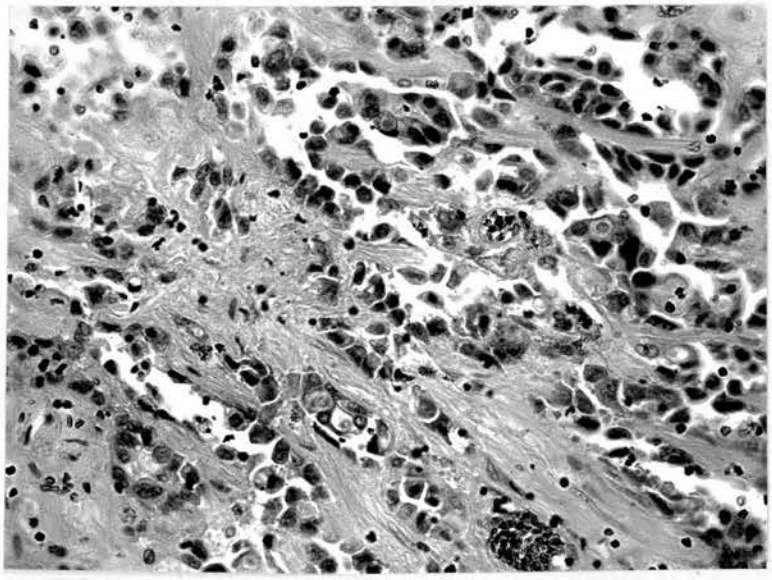

Fig.31,(Case 11.) : High power of Fig.30, showing phagocytosis of red blood cells. x 250.

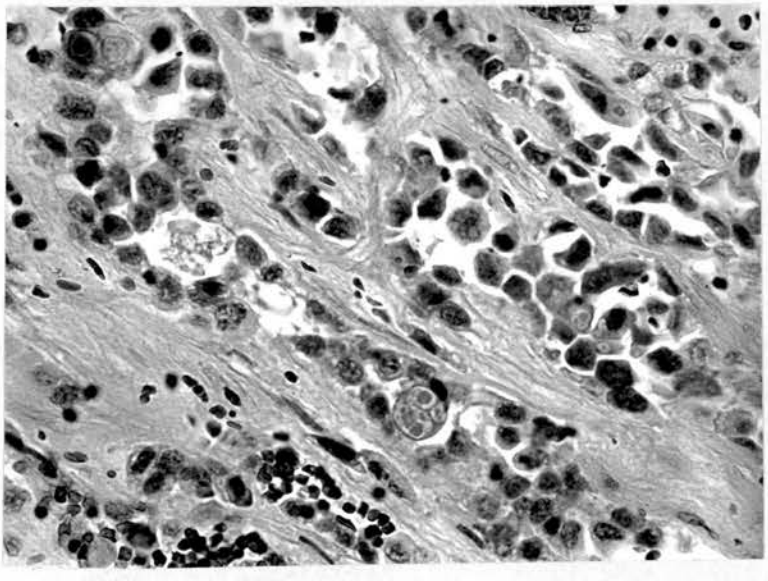

Fig.32,(Case 11.) : Same case as above showing phagocytosis of red blood cells. x 375.

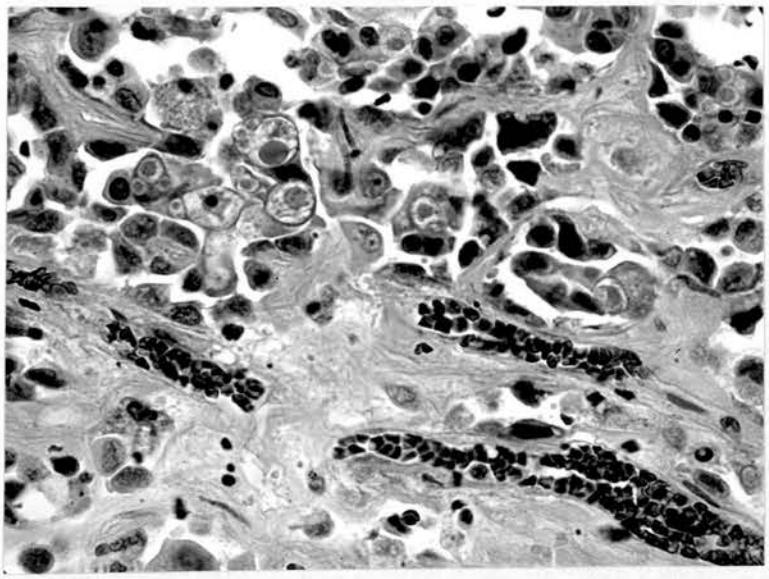

Fig.33,(Case 11.) : Phagocytosis of red blood cells in regenerating lung tissue of an organised infarct. x 375.

SECTION II

(PART I)

REPARATIVE CHANGES IN LUNGS REMOVED SURGICALLY IN TUBERCULOSIS AND BRONCHIECTASIS

REPARATIVE CHANGES IN LUNGS REMOVED SURGICALLY IN TUBERCULOSIS AND BRONCHIECTASIS

INTRODUCTION.

The hyperplasia of the alveolar wall in the region of the healed infarcts of lung is not unique. Similar reaction to injury has been noted in bronchiectatic lesions, infectious granuloma, atelectasis, chronic bronchitis and focal fibrosis, as well as infarcts. (King; Raeburn & Spencer, 1953; Peterson et al, 1949).

In "Acute diffuse interstitial fibrosis of lung", a clinical and pathological entity of unknown aetiology, commonly known as "Hamman-Rich Syndrome", was first described by Hamman and Rich in 1935. Later, Hamman and Rich (1944) reported four cases of diffuse interstitial fibrosis of lungs conforming to a distinctive histological pattern, but not attributable to any known cause. Since then increasing number of cases have been reported which fulfilled thehistological criteria of Hamman-Rich. (Potter & Gerber, 1948; Golden & Tullis, 1949; Katz & Auerback, 1951; Grant & Associates, 1956; Carabasi, 1958). According to Carabasi/

Carabasi less than 65 cases of diffuse interstitial fibrosis (Hamman-Rich Syndrome) have been reported by various authors. In the most classic stage of the syndrome, as gathered from the reported cases, the alveolar septa are thickened by cellular connective tissue. Mononuclear cells and fibroblasts are present in various proportions, and the alveolar spaces are lined by a continuous layer of prominent cuboidal epithelium. The capillaries of the alveolar walls are described by most authors as dilated, though some have found them collapsed and bloodless. Increased deposition of connective tissue leads to gross thickening of alveolar walls and reduction in size of alveolar spaces, which contain fewer cells than previously. Should the patient survive for a sufficient time collagen is deposited in the alveolar walls, which become more dense, less cellular, and somewhat thinner. Hyperplastic alveolar epithelium and alveolar capillaries are now absent (Read, 1958).

It is not uncommon to see alveoli in contact with a scar lined by a single layer of plump cuboidal cells. Atypical proliferation with epithelial-bud formation, squamous metaplasia/

plasia and spaces lined by cubical or flattened epithelial cells within the areas of healed tuberculous lesion or any fibrosed part of lung tissue can often be seen during routine examination of sections of lung. In order to determine whether this group of cases followed the same general pattern of repair, some hundreds of biopsy sections of lung tissue, containing old tuberculous and bronchiectatic lesions were examined.

MATERIALS AND METHODS

About 300 cases were selected for microscopical examination from the biopsy material of lung tissue containing old tuberculous and bronchiectatic lesions. In some cases fresh sections were prepared from paraffin blocks and stained with haemotoxylin and eosin, Hart's elastic tissue stain and for iron (Prussion blue reaction). The majority of the sections revealed almost similar microscopic picture, consisting of fibrotic proliferation of the lung parenchyma, epithelial hyperplasia of bronchiolar epithelium with cuboidal cell lining of alveolar walls. As a representative group 8 such cases are discussed below. The diagnosis of/

of each case was established clinically and confirmed microscopically. Three of them are old tuberculosis and the other five are lon-standing bronchiectasis.

CASE REPORTS

Case 1.

Male, 22 years. Duration of illness - several years. Thoracotomy was performed in belief that he had tuberculosis of apical segment of lower lobe, but in fact he was found to have a non-tuberculous purulent cyst in the posterior segment of upper lobe.

The specimen consisted of 3 separate pieces of lung tissue, the largest being 6 x 2½ cm. which embody several small communicating cyst-like areas lined with firm fibrous tissue and containing necrotic debris. Part of the wall is cartilage. Microscopically, the largest specimen shows firm fibrous tis-:sue containing many tortuous dilated bronchi, some with areas of squamous metaplasia of the mucosa. There is considerable infiltration of inflammatory cells, mainly lymphocytes and plasma cells both in the bronchi and surrounding tissue. No aerating alveolar tissue is present. Some areas of necrosis are seen, but/

but no typical tuberculous zone. Diagnosis: bronchiectasis following pulmonary fibrosis.

Fig. 34 is the photomicrograph of one of the areas of a section of the larger piece of lung. Figs. 35 & 36 are high power view of Fig. 34. Low power view of the section shows fibrotic zone almost all over the field. One of the dilated bronchi is seen filled up with necrotic material. The narrow spaces which are seen amidst the fibrous tissue seem as if they were invading all round into the fibrotic zone. The penetrating ends are cords of regenerating bronchiolar epithelium growing into alveoli. Most of them are lined chiefly by cuboidal cells, a few by columnar epithelium. (Fig. 36).

Case 2.

Male, 34 years. Cough and sputum all throughout his life. Bronchogram showed gross left lower bronchiectasis with saccular dilatation. When removed the left lobe was seen covered with thick pleura except in the fissure, where lung tissue looked quite healthy. The undersurface of the lobe looked bluish and felt rubbery. Very large glands at the hilum with big bronchial vessels. Resection of the left/

left lower-middle lobe performed.

This specimen is the resected left lower lobe of lung, and shows considerable degree of aeration; the pleural surface is grossly thickened. At the periphery of the lung there are many small yet dilated bronchi with thickened walls filled with mucus and pus. The surrounding lung shows alternately emphysema and atelectasis. Microscopically, the sections of lung show many dilated bronchi with peribronchial fibrosis and chronic inflammatory cell infiltration of their mucosa. The columnar lining cells are hyperplastic and project, in some instances, into the bronchial lumen in papilliform processes. The mucous glands are increased in number and are actively secreting mucous. Numerous lymphoid follicles are present in close proximity to the dilated bronchi and many proliferating cubical epithelial cells are present in an around these follicles. The surrounding lung is emphysematous, atelectatic, fibrotic, with some areas of bronchopneumonia. There is endarteritis obliterans. The pleura is thickened and fibrosed. The appearance is that of follicular bronchiectasis with areas of atelectasis and emphysema around the bronchi.

Figs./

Figs. 37 & 38 illustrate the bronchial changes with a representative area of alveoli lined by cubical epithelium in the inflammatory tissue. The epithelial buds are seen penetrating into the area of fibrosis to form new bronchi and bronchioles. (Figs. 38, top left).

Case 3.

A 14 year old boy, had whooping cough at 8½ months with repeated attacks of respiratory infection since. Green sputum with cough present since boy-hood. Bronchogram showed gross lingular bronchiectasis. The lingula was resected. It was airless and very small.

The specimenconsists of resected lingual. The pleural surface shows one or two fibrous adhesions and several areas of depigmentation. The lung is firm and elastic in consistence. On section several large bronchi show well-marked peribronchial fibrosis and filled with thick watery pus. The surrounding lung has focal emphysema. Microscopically, there are dilated bronchi lined by hyperplastic columnar epithelium deep to which are many proliferating lymphoid follicles. There is considerable peribronchial/

peribronchial fibrosis with small areas of resolving bronchopneumonia around the bronchi. The surrounding lung shows emphysema. Many alveoli also have fibrous-thickened walls and many proliferating bronchioles are seen in relationship to lymphoid follicles. Diagnosis is follicular bronchiectasis.

Figs. 39, 40 & 41 are typical of the reaction of the bronchial and alveolar epithelium.

Case 4.

This 9-year-old boy had pneumonia at age 3 with cough since. Bronchogram showed bronchiectasis in the lingula and at operation an atelectatic lingula and at operation an atelectatic lingula was removed. The rest of the lung was healthy.

The specimen as received showed many dilated bronchi from which thin watery pus exuded. Considerable degree of peribronchial fibrosis was present. Microscopically, the dilated bronchi are lined by hyperplastic columnar epithelium; their lumina are filled with inflammatory debris. Their walls infiltrated with lymphocytes and contain hyperplastic lymphoid follicles. The lung contains hyperplastic lymphoid follicles and proliferating bronchiolar epithelial/

epithelial cells as in follicular bronchiectasis.

Epithelial proliferation and bud formation are clearly visible in many parts of the section. Regeneration of alveoli in the subpleural region with necrotic debris in their cavities are seen. (Fig. 42). The new alveoli are atypical in shape and are lined by cubical cells. Fig. 43 shows such alveoli (lower part) and, in the upper part of the figure, small bronchioles with epithelial proliferation.

Case 5.

10-year-old boy had nasal discharge and productive cough (blood-stained) since childhood. At operation a shrunken solid middle lobe was removed. Microscopically, there are dilated bronchi lined by hyperplastic swollen columnar cells, in some cases forming papilliform processes. There are large hyperplastic lymphoid follicles in the mucosa and sub-mucosa. The surrounding lung is atalectatic. Many alveoli are infiltrated with lymphocytes. Some alveolar spaces are lined by cubical epithelium.

Dilated bronchi with thick walls are seen in/

in Fig. 44 which shows aerated spaces almost all over the field. On high power view these spaces are seen lined by cubical and some by flattened cells. (Fig. 46). These are regenerating bronchioles giving rise to alveolar formation. One of the smaller bronchi in the lower part of the field shows columnar cells becoming cubical and flattened epithelium growing into alveoli. Fig. 45 is an area of regenerating lung tissue near a dense fibrous area.

Case 6.

Female, 27 years, suffered from tuberculosis for 8 years. The right upper and middle lobes, apical lower and antero-medial basal segments were removed.

The specimen showed chronic tuberculosis with cavity formation. Microscopically, sections from all the pieces of lung show the picture of chronic fibro-caseous tuberculosis, with tuberculous granulation tissue. The surrounding lung is fibrosed, infiltrated with chronic inflammatory cells and contains tuberculous follicles.

Epithelial cell proliferation with regenerated bronchi and alveoli are conspicuous in many parts of the fibrosed area. Figs. 47 & 48/

48 show regenerating lung with bronchial tissue. Epithelial cell proliferation, atypical alveolar regeneration are quite evident in these areas. (Fig. 48). The newly formed bronchi and alveoli are lined by cubical and flattened cells.

Case 7.

Female, 22 years, suffered from pulmonary tuberculosis for one year. She was treated surgically with removal of the left apico-posterior and anterior segments. The specimen consists of the apico-posterior and anterior segments of lung. Microscopically, sections from different parts show chronic fibro-caseous tuberculosis with fibrous-walled cavities lined by some necrotic debris and fibrous tissue but devoid of active epithelioid tissue and giant cells. The rest of the tissue shows moderate fibrosis with alveoli lined by cubical epithelium, chronic inflammatory cell-infiltration and a few scattered regressive tuberculous foci surrounded by fibrous tissue.

The fibrous areas are invaded by regenerating epithelial buds from all sides. (Figs. 49 & 50). Regeneration of bronchi and alveoli are conspicuous in these areas. Hyperplastic lymphoid/

lymphoid follicles are seen and they are pervaded by the proliferating epithelial cells with their penetrating buds. (Fig. 51). The regenerating alveoli are lined by cubical cells mostly but some are seen to have typical alveolar cell lining. (Figs. 51 & 52).

Case 8.

Female, 29 years, suffered from tuberculosis for 10 years during which she has had intermittent treatment of collapse and chemotherapy. The left lung underwent destruction with gross cavitation and pneumonectomy was performed.

The specimen consists of the left lung which has been largely replaced by dense fibrous tissue. There is considerable peribronchial thickening and in the apex there is a well-circumscribed cavity with dense walls. Small firm areas of old caseation are scattered throughout the anterior surface of the lung. Microscopically, most of the normal lung is replaced by dense fibrous tissue with collections of lymphocytes among which are epithelial channels. One or two caseating foci are seen along with chronic fibro-caseous tuberculosis.

Lymphocytic proliferation together with epithelial/

epithelial bud formation in the fibro-caseous area are seen in abundance. (Fig. 53). The buds are prominent and are lined by cubical cells, but the invading end of the buds sometimes consists of masses of cells. (Fig. 54).

SUMMARY AND DISCUSSION

The material from 5 cases of bronchiectasis, and 3 of old tuberculosis of human lung, reveal evidence of regeneration of lung parenchyma during their reparative phase. The findings resemble those of infarcted lung in the pattern of their reparative changes. It is noteworthy, however, that the activity of the regeneration is very considerable in bronchiectasis in young people, the so-called follicular type of lesion. Here there are abundant examples of bronchial budding and of alveolar spaces opening and re-forming in the peribronchial inflammatory tissue. The direction of the newly-formed air spaces is towards the consolidated lung and by serial sections, the communication with the bronchus is readily established.

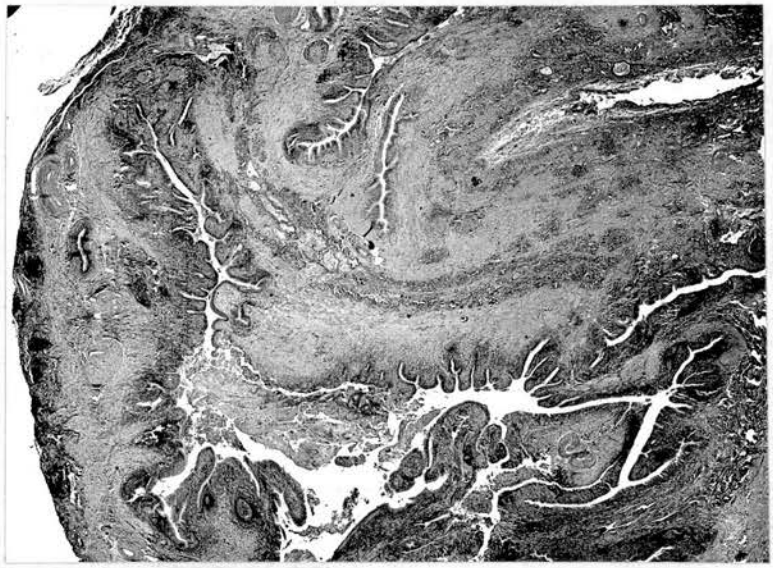

Fig.34.(Case 1.) : Bronchiectasis following fibrosis. The fibrous zone is invaded by hyperplastic epithelium of bronchi and bronchioles. One big dilated bronchus is seen in the field giving rise to epithelial buds. x 8.

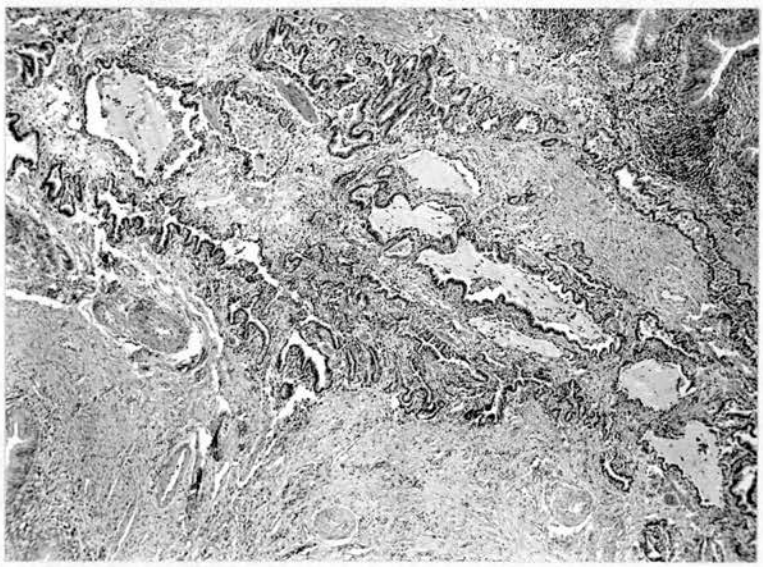

Fig.35.(Case 1.) : High power view of the above field showing the bronchial epithelial regeneration. x 40.

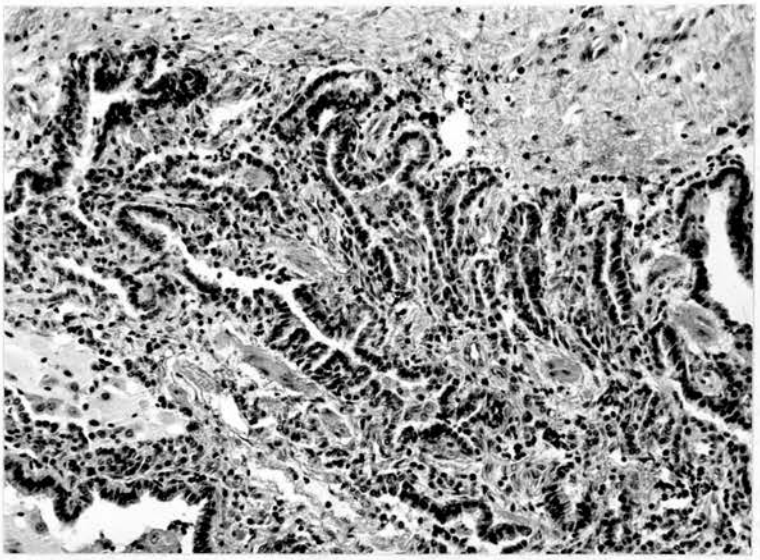

Fig.36.(Case 1.) : Higher power view of Fig.35, showing cuboidal and columnar cells lining the regenerating bronchial buds growing into alveoli. x 140.

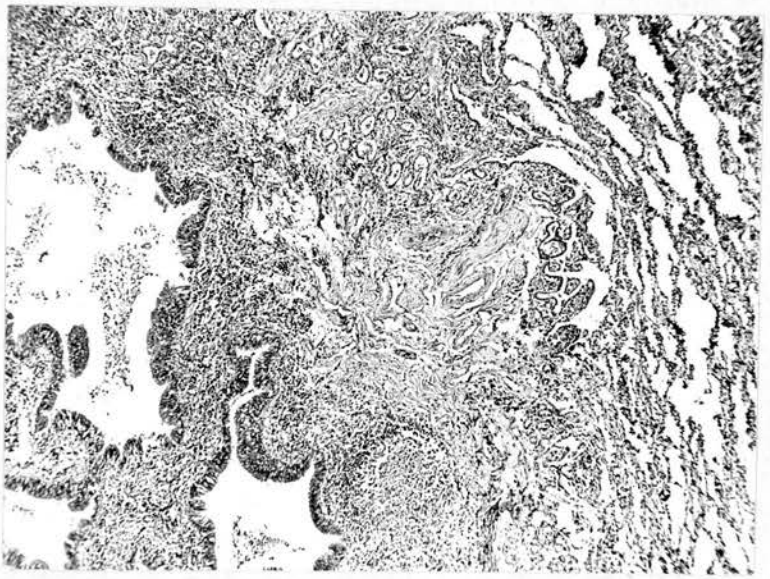

Fig.37.(Case 2.) : Low power photomicrograph of a bronchiectatic lung to show bronchial buds and alveolar spaces in the peribronchial inflammatory tissue. x 40.

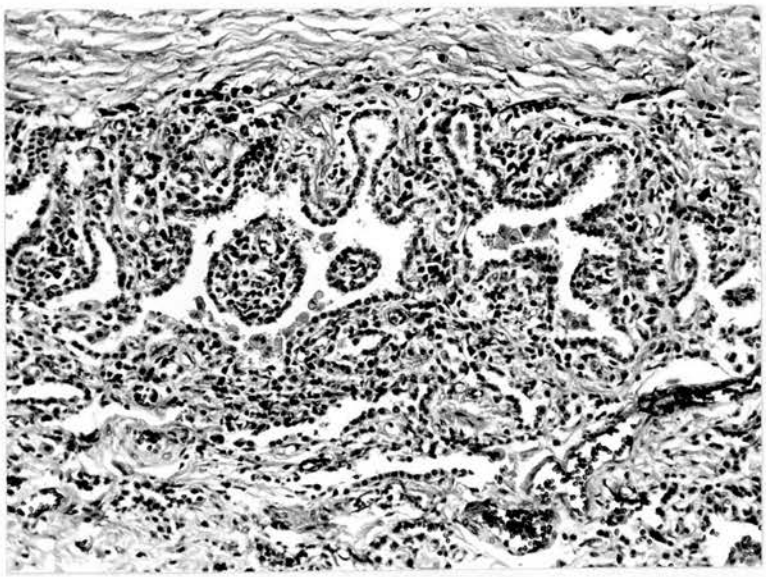

Fig.38,(Case 2.) : Alveolar spaces in process of re-formation in chronic inflammatory tissue. x 135.

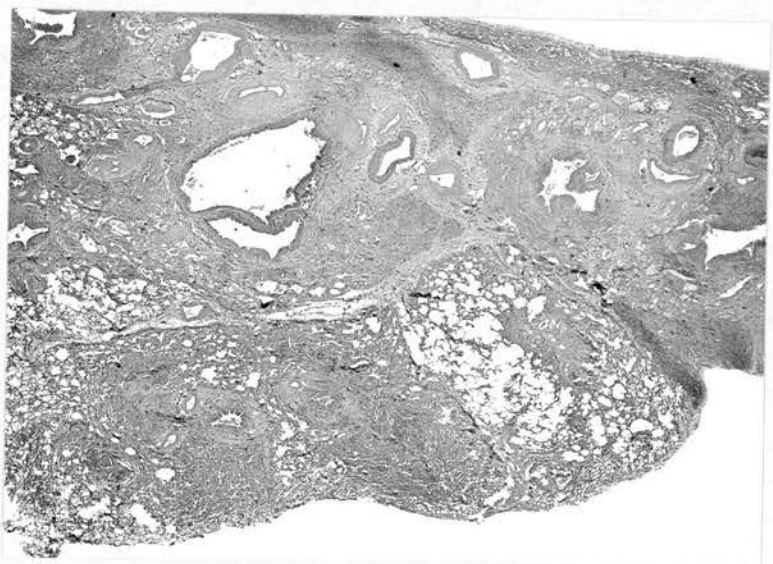

Fig.39,(Case 3.) : Low power photomicrograph to show dilated and inflammed bronchi with peribronchial fibrosis and focal emphysema. x 8.

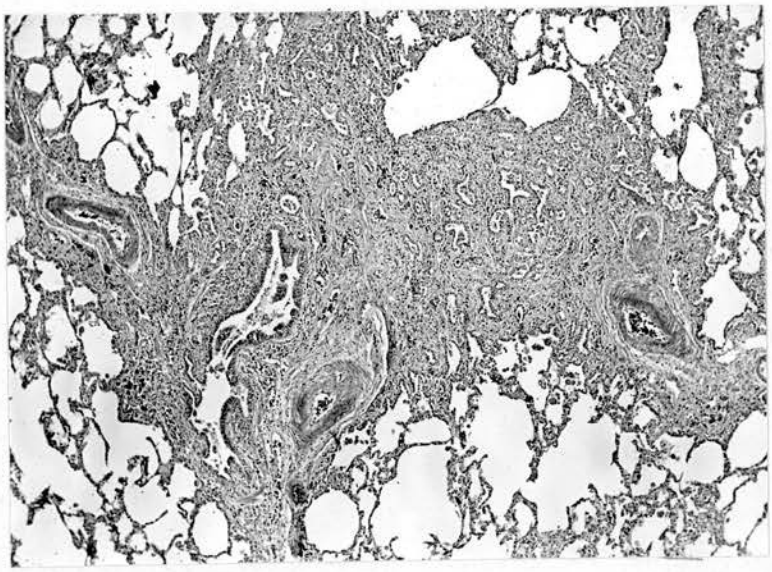

Fig.40,(Case 3.) : High power photomicrograph of a fibrotic collapsed area with alveolar spaces re-opening or re-forming. Emphysema is also seen. x 40.

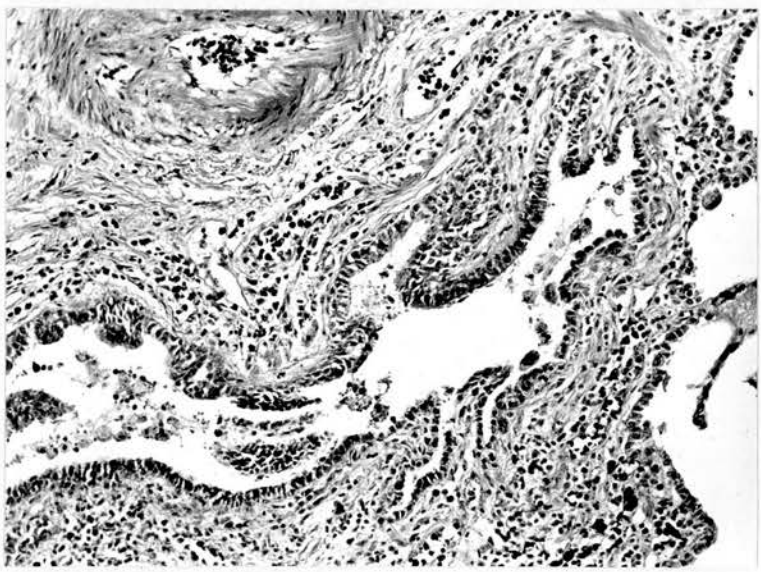

Fig.41,(Case 3.) : The cubical lining of the bronchial buds is well shown. x 135.

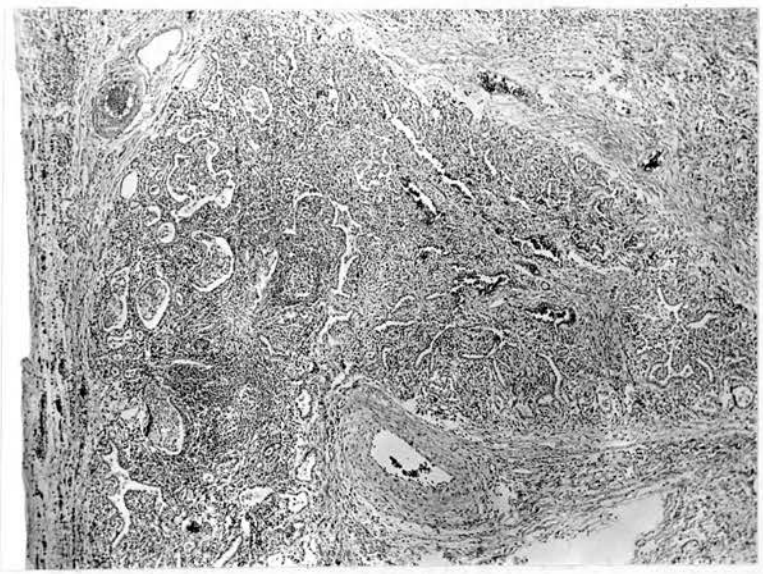

Fig.42,(Case 4.) : Alveolar spaces and bronchial buds forming in subpleural repair of bronchiectatic lung. x 40.

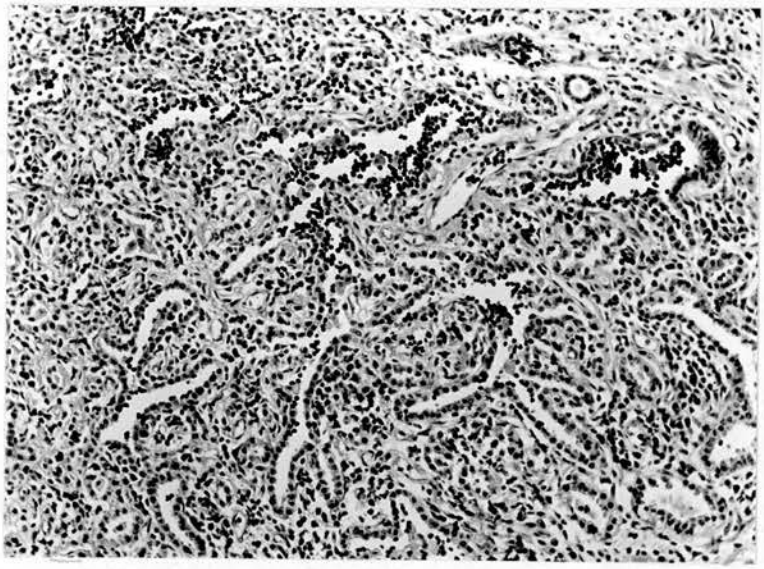

Fig.43,(Case 4.) : Higher power photomicrograph to show detail of new air spaces, lined by cubical cells, in inflammatory lung tissue. x 135.

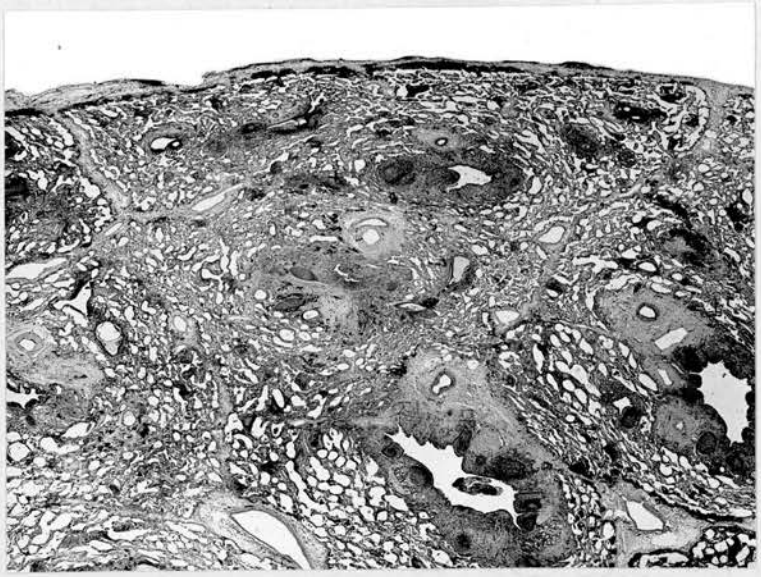

Fig.44,(Case 5.) : Bronchiectatic lung to show peribronchial infiltration. x 8.

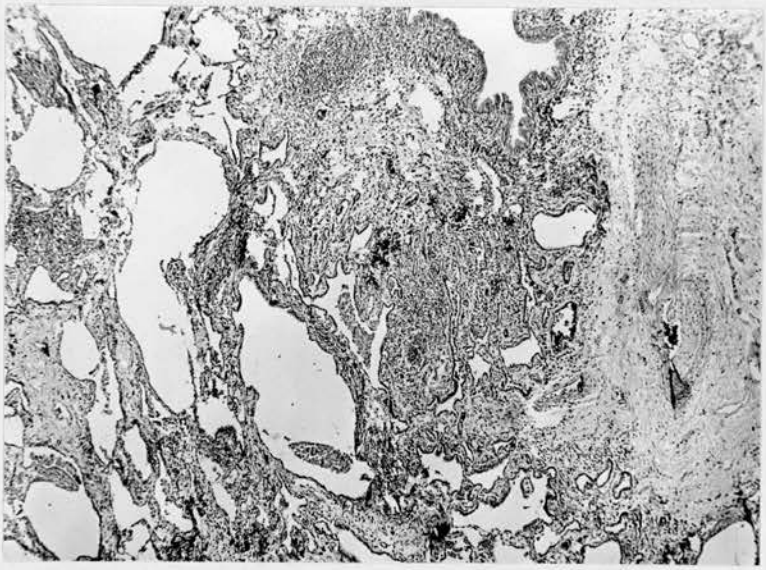

Fig.45,(Case 5.) : Bronchial buds opening and leading into consolidated lung where new air spaces have formed. x 40.

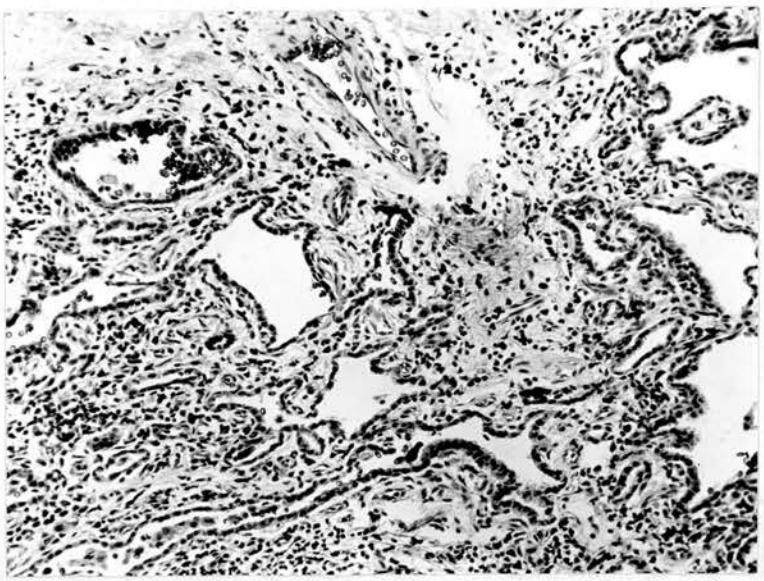

Fig.46,(Case 5.) : Higher power photomicrograph of Fig.45, to show bronchial buds and new air spaces lined by cubical cells. x 135.

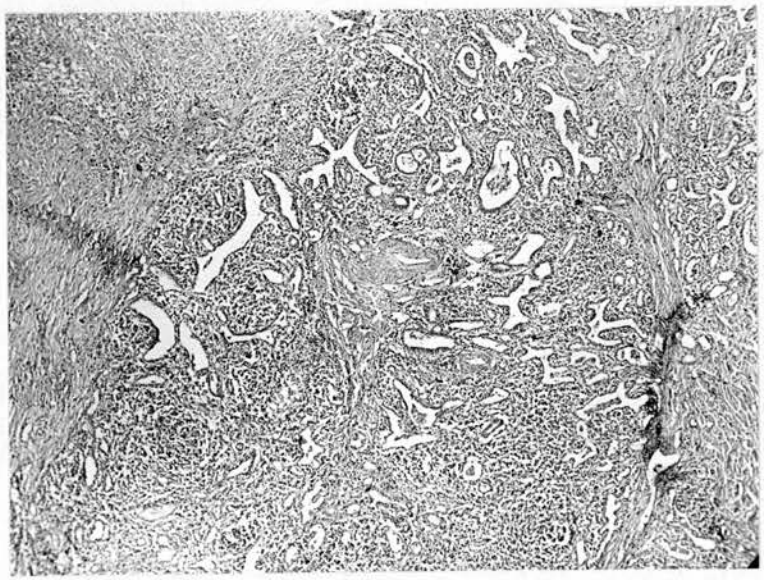

Fig.47,(Case 6.) : Bronchial buds and reformed alveolar spaces in connective tissue around the caseous nodules. x 40.

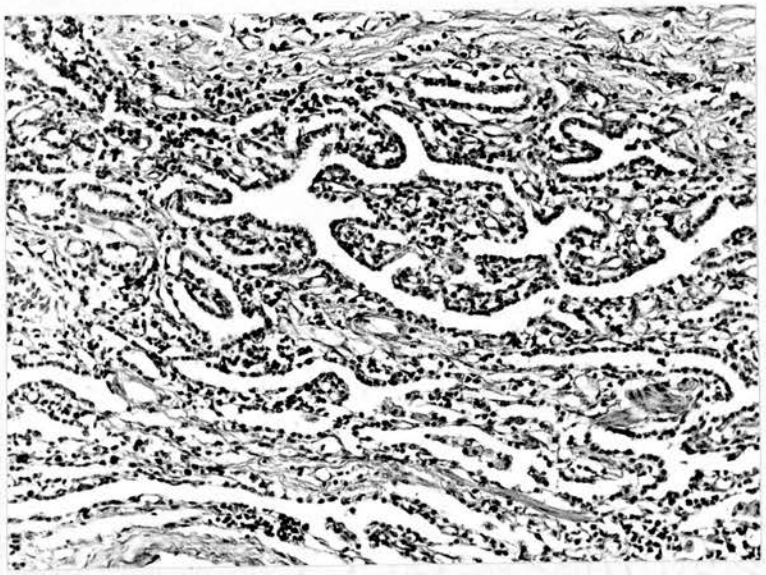

Fig.48,(Case 6.) : Higher power photomicrograph of Fig.47, to illustrate detail. x 135.

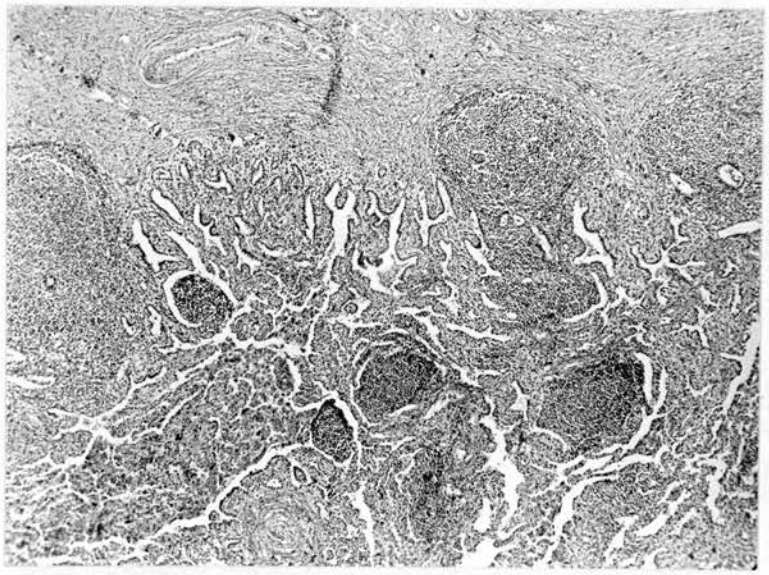

Fig.49,(Case 7.) : Air spaces with cubical epithelium extending towards the fibro-caseous tissue. x 40.

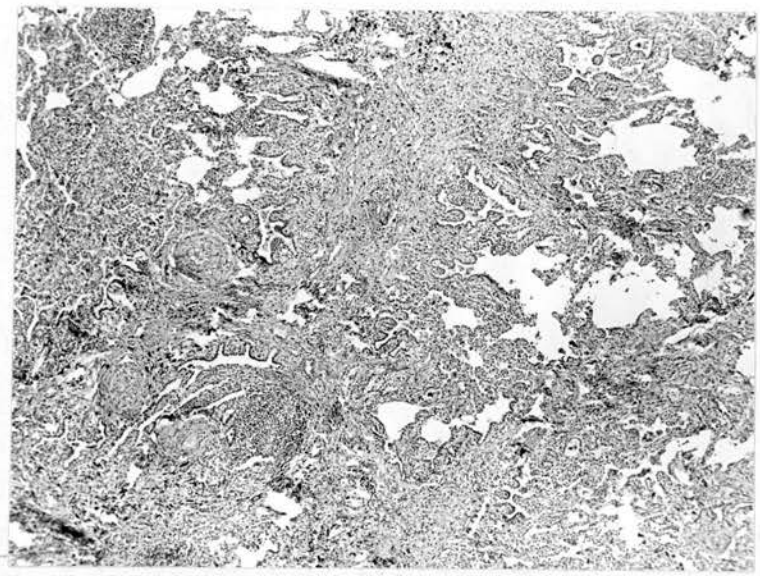

Fig.50, (Case 7.) : Low power photomicrograph to show the formation of air sacs and bronchial buds. x 40.

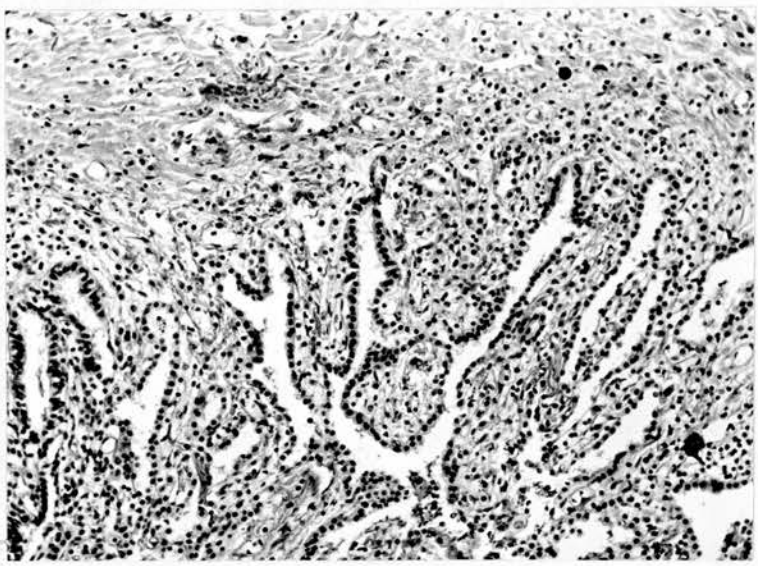

Fig.51,(Case 7.) : Higher power of the above figure to show detail of new alveolar spaces. x 135.

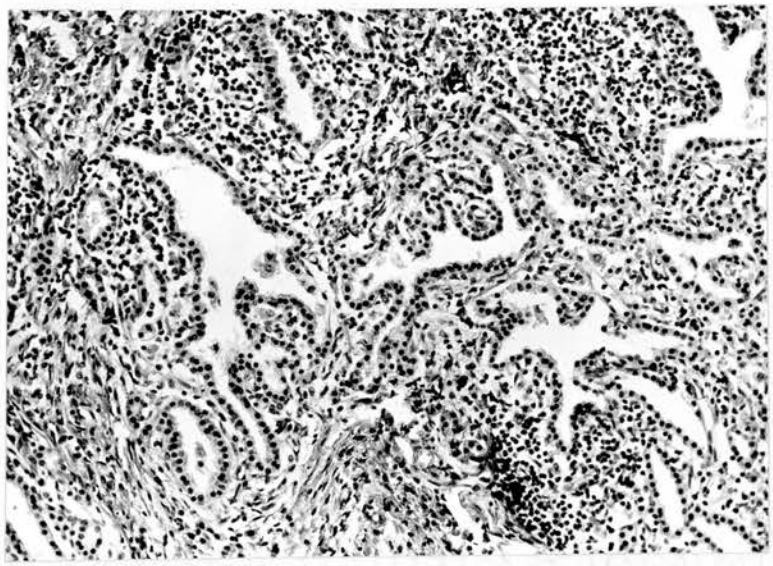

Fig.52,(Case 7.) : High power of Fig.50, to show the regenerating area of lung. x 135.

Fig.53,(Case 8.) : Low power field to show dilated air spaces and bronchial buds. x 40.

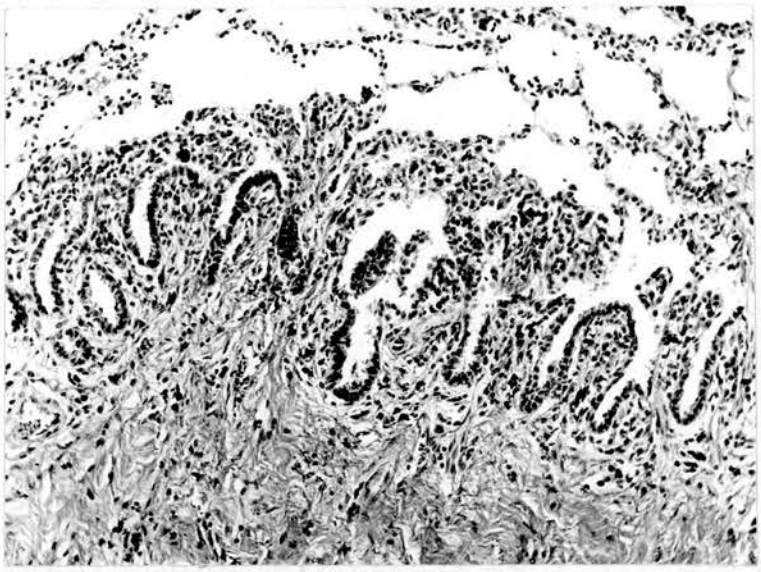

Fig.54.(Case 8.) : Re-formation of alveoli in fibrous connective tissue from a case of fibro-caseous tuberculosis. x 135.

PART II

REGENERATION OF TRACHEAL EPITHELIUM IN RATS

REGENERATION OF TRACHEAL EPITHELIUM

INTRODUCTION.

The regeneration of tracheal epithelium must be a relatively common process, to judge by the frequency of acute tracheitis in upper respiratory infections. For obvious reasons, this is not capable of controlled experiment, though Winternitz (1920) and Winternitz et al (1920) made observations on human material and experimentally studied the regeneration of tracheal epithelium in animals exposed to chlorine gas. Other experimenters have used the traumatic approach. Using rats, anaesthetised with ether, Condon (1942) removed portions of tracheal epithelium through a tracheotomy opening by means of modified iridectomy forceps and a small scalpel. Wilhelm (1953) used closed technique and removed small portions of the mucosa endo-tracheally by a small aural curette in rats. Both studied the regenerative process of the epithelium of the tracheal mucosa.

It was decided that the regeneration of the tracheal mucosa should be re-examined mainly as an introductory exercise to the experimental study/

study of lung repair and also to determine whether the application of histo-chemical methods would be of value.

EXPERIMENTAL PROCEDURE

40 Albino Wister rats of approximately 120-160 g. body weight were used. The animals were kept on normal diet during the whole period of experiment. The operative procedure chosen was similar to that of Wilhelm.

Under ether anaesthesia and with strict asepsis the trachea of the animal was exposed by a midline incision in the neck for fixation. With a small modified aural curette, sterilised in alcohol, the mucosa of the anterior wall of the trachea was curetted endotracheally from just beyond the thoracic inlet to the lower margin of the larynx. Exposure of the trachea, without opening it, allowed the curetted site to be identified accurately and an even pressure to be maintained on the instrument so that the curetting could be kept steady and regular in depth. Care had to be taken not to curette too deeply so that the elastica of the basal layer of the mucosa remained undamaged. The opening/

opening of the skin in the neck was closed by 2 or 3 cotton sutures and healed without disturbance.

Unfortunately, 5 animals died during the operation and 4 were lost from acute respiratory infection within a few days of operation. The remaining 31 rats were killed at intervals of 1 hour - 45 days after operation. Haemorrhage after operation was negligible. The animals usually returned to their normal state within 4 - 5 hours and by 24 hours they seemed to be quite normal. The external wound of the skin of the neck healed completely by a week or so.

The lower end of the trachea of each animal was cut transversely into several small pieces after removal and was fixed in suitable fixatives for different staining method, viz., in 10 per cent buffered formol-saline for haemotoxylin and eosin, alcian-blue reaction, elastic tissue stain and for P.A.S.; in modified Bakaer's fluid for ribonucleic acid and desoxyribonucleic acid; in cold acetone (4°) for enzyme reactions. After paraffin embedding transverse sections of these pieces from different fixatives were cut at 5u thickness and

were/

were stained with Ehrlich's haemotoxylin and eosin, Hart's elastic tissue stain, periodic-acid-Schiff method of de Tomasi and with alcian blue method for mucopolysaccharide, Kurnick's plasma cell stain for R.N.A. and D.N.A., and for acid and alkaline phosphatase and non-specific esterase reactions the sections were stained by modified Gomori's methods. Detailed description of the various staining techniques is given in the chapter of the appendix.

OBSERVATIONS AND RESULTS

Microscopical examination of sections of tissues removed at intervals of 1 - 4 hours after the production of injury showed haemorrhage into the injured area, dilatation of the surrounding blood vessels, and the formation of fibrin-clot upon the surface of the denuded area. (Figs. 55 & 56). Submucosal oedema soon made appearance, and the infiltration of mononuclear cells into the surrounding areas was prominent. After an hour effused blood and loose fibrin-clot containing entangled cells and leukocytes appeared over the denuded surface. At the end of 4 hours the epithelial cells of the basal layer of the mucosa were seen to/

to have changed their shape from columnar to flattened cells. After longer intervals of time, such as 6 - 8 hours, there was evidence that organisation of the haemorrhage had started and the first sign of epithelial cell regeneration was observed. (Figs. 58 & 59). This consisted in the formation of flat pavement-like cells overlying the normal basal cells at the extreme periphery of the injured area. Between 4 - 6 hours the intact epithelial cells at the margin flattened out (Figs. 57 & 59), lost their cilia and spread laterally over the surface, in close association with the elastic lamina. Almost in all sections, quite a number of marginal ciliated columnar cells exhibited this differentiation and they alone migrated. At first these migrating cells showed tuft of cilia (Fig. 59), but cilia could not be seen after 20 - 24 hours when the epithelial cells had become flattened.

Regeneration of epithelium and simple stratification of the new epithelium

By 10 - 24 hours the inflammatory reaction had spread out. Steady progress occurred in the local reaction, fibrin-clot became firmer and deeper, the submucosal exudate increased in amount/

amount and in neutrophil content and soon involved the peritracheal tissue by spreading around the margins of the curettage. All specimens taken 10 hours after injury showed a similar degree of epithelial migration. The extent of regeneration at any interval following the operation mostly depended upon the depth of injury and the amount of inflammatory reaction which had occurred. In animals killed 10 - 12 hours after injury, epithelium had regenerated in a characteristic manner. Spreading from the periphery of the injured area out over the denuded surface, which at this time was essentially an immature granulation tissue, the epithelium consisted of two-cell deep layer by the end of 12 hours and of many layers of flat deeply-staining cells by 24 hours. (Figs. 61 & 62). The regenerating marginal cells invaded the wound from both ends to cast off the fibrin-clot which covered the denuded surface. (Fig. 60 & 63). Mitosis in the new cells was very inconspicuous at the early period. One or two mitotic figures in the basal cells were found by 24 hours after injury in the unaffected part of the epithelium of the trachea. (Fig. 64).

Curetted/

Curetted necks of submucosal glands also contributed to the progress of spreading cells, and often formed independent lines of extremely flattened cells under the fibrin-clot. (Figs. 60 & 61). By 20 - 24 hours the epithelial cells had spread quite a great distance almost covering two-thirds of the injured area (Figs. 62 & 63). The advancing epithelial cells were always in close application to the intact elastic lamina. A narrow clear zone could often be seen around and immediately ahead of the line of spreading cells as if these were producing a fibrinolytic ferment which cleared the path of their progress. (Fig. 60).

The peak of inflammation was reached in 24 - 36 hours. By 72 - 96 hours neutrophils and oedema were decreasing in amount and almost the whole injured area was covered up by newly-grown epithelial cells, two to three layers deep. (Figs. 66 - 68).

The fibrin-clot generally broke away at 48 - 72 hours when the spreading margin of epithelium undermined it but where the elastic lamina was breached final separation might take up to 4 - 5 days (Fig. 70). Provided that the elastic lamina was not damaged (Fig. 72), the/

the curetted surface was covered by a continuous line of simple cells and the separated fibrin-clot was shed within 48 - 72 hours. (Figs. 65 & 66). The new covering was 2 - 3 cells deep at its margin and the two approaching lines of cells from both ends met without any over-riding but whereas, in some animals, killed at the same intervals where severe inflammatory reaction was present, the epithelial regeneration had progressed to only a slight degree. Instances were found in sections where the submucosa remained firmly packed with inflammatory products, swollen with oedema, the epithelium covering this area remained thin and consisted of only 2 or 3 layers of deeply-staining cells with large nuclei. There was no evidence of the formation of multiple layers or of the production of cilia or mucosa. (Fig. 71).

Re-differentiation of new epithelium

From the fourth day onwards sign of re-differentiation of the new epithelium became evident. In these cases the elastica of the mucosa was not breached and there was very little inflammatory reaction. The transitional epithelium/

epithelium started to arrange itself into layers of lower cuboidal cells with an underlying basal zone of flattened cells. The top layer of these transitional cells were producting minute cilia. (Fig. 69). By the end of the fifth day, the re-differentiation was well established, and within 6 - 7 days the new cells were often low columnar type.

Sections from animals killed at intervals of 5 - 6 weeks showed complete regeneration and re-differentiation of epithelium lining the trachea over the injured surface. They showed a thin row of flat elongated cells which rested upon the peripheral fibrous strands of the sub-mucosa. Resting upon these flat basal cells was a thin layer of columnar cells with narrow centrally placed nuclei. The distal ends of these columnar cells were equipped with tiny cilia (Fig. 82). Goblet cells were seen in sections of animals killed 14 - 15 days after operation. (Fig. 76).

Though in one or two cases cilia were seen as early as 4 days after curettage, (Figs. 69), ciliated cuboidal or low columnar cells more often appeared between 15 - 21 days of operation, (Fig. 75 & 76) and were constant by 4 - 5 weeks. Complete re-differentiation required about/

about 30 - 45 days. (Figs. 78, 80 -82). The regenerated epithelium thus consisted of flat basal cells, topped by cuboidal or low columnar cells, the cilia of which in places were irregular, but nevertheless present.

In some preparations, however, even after 2 - 3 weeks after operation, reversion to the normal epithelium had been accomplished slightly. In these sections either the elastic lamina of the mucosa was damaged or the sub-mucosa was filled with inflammatory exudates, the overlying epithelium of which still remained in an irregularly stratified form. There were no cilia or goblet cells in the mucosa. (Figs. 73 & 74). Chronic inflammatory reaction was found in the sub-mucosa of these sections. In Fig. 77 proliferation of elastic tissue was seen in a part of the area of deep injury in an animal killed 21 days after operation where the elastica was breached. The injured surface was covered with a layer of flattened cells only. The elastic lamina, however, became completely reorientated in 4 - 5 weeks. (Fig. 79).

HISTOCHEMICAL REACTION OF REGENERATING EPITHELIUM

In/

In this part, the results of a histochemical study of enzymes, viz., acid and alkaline phosphatase, and non-specific esterase; R.N.A. and D.N.A. and of mucopolysaccharide of the regenerating epithelium of curetted tracheal mucosa is presented. This investigation was undertaken as an effort to demonstrate a histochemical reaction pattern in cells of the regenerated epithelium which would be distinctive from those occurring in normal mucosa.

Normal Mucosa: Normal tracheal epithelium of rat showed enzymic activity, admittedly of a strong nature. Figs. 84, 85 and 86 show the epithelial cells of the mucosa giving positive staining reactions for non-specific esterase, acid and alkaline phosphatases respectively. Non-specific esterase and alkaline phosphatase activities are seen in the supra-nuclear region and that of acid phosphatase in the nuclear region.

Similarly the goblet cells of the normal tracheal mucosa gave positive staining reactions to alcian blue and P.A.S. for mucopolysaccahride in the supranuclear region of the cell. (Fig. 87, 88).

As far as could be demonstrated by R.N.A. and/

and D.N.A. staining reactions the cells of normal tracheal epithelium showed their presence by fairly strong activity in the nuclear region. (Fig. 89).

Regenerating Epithelium: It was observed that within 48 - 72 hours of curettage the flat migrating cells of the regenerating mucosa gave positive phosphatase reactions which were weaker than the normal. (Figs. 90, 91). These reactions became fairly strong in regenerated cells 1 or 2 weeks old (Fig. 92) after which, though the alkaline phosphatase reaction remained strongly positive acid phosphatase became less intense in regenerated cells till they are fully differentiated.

Non-specific esterase reaction was mildly positive in flat migrating cells in the early stage of regeneration. (Fig. 93). By second or third week the regenerating cells gave fairly strong reaction for esterase. (Fig. 94). This reaction was observed to reach almost normal intensity in fully differentiated epithelial cells in 5 - 6 weeks. (Fig. 95).

Similarly the reactions for R.N.A. and D.N.A. in the migrating cells was clearly less intense than normal (Figs. 96, 97). This weakened/

weakened R.N.A. and D.N.A. reactions persisted for several days, (Fig. 98), and did not return (Fig. 99) to normal intensity till the cells were fully matured.

Alcian blue reaction for mucopolysaccahride was negative in the early regenerated cells but in the latter stage, the fully differentiated mucous-secreting cells of the mucosa gave positive reaction to alcian blue. (Fig. 100).

DISCUSSION

My experimental work confirms the findings of Condon and Wilhelm. Condon reported that normally the tracheal epithelium was slowly regenerative and under conditions of stress, this epithelium might alter its form and show rapid regenerative process; the rapidity of such regeneration was dependent upon the quality of the stroma across which the epithelium must grow. The healing process was essentially a function of the basal cells which form the initial layer. But my observations agreed with the experimental results of Wilhelm who reported that the initial layer of epithelium, to cover the denuded surface, was derived from the ciliated columnar cells as migrating flattened epithelium which was evident from the fact that these cells contained cilia during the earliest stage of migration. Condon also studied the effect of colchicine in the tracheal epithelium in a group of animals followed by excision of limited area ofthe mucosa. Colchicine was used as the static agent to hold mitosis in the metaphase for a given period. Wilhelm observed a certain periodicity of mitosis in colchicinized animals. After curetting the/

the tracheal epithelium two waves of mitotic activity were apparent in the old and new epithelium respectively, being well demonstrated in animals treated with colchicine. The first wave occurred 24 hours after curettage in the paramarginal old epithelium, at the same time a few mitoses could be seen in the lines of spreading cells. This proliferative activity had subsided in 48 hours. At 72 hours, mitoses were frequent in thenew epithelium specially when submucosal inflammation was pronounced.

Although I did not use colchicine, my findings also indicate a double wave of mitotic activity. I believe the migratory process of the epithelium is a physical process where the cells, while retaining connection with one another by their cytoplasmic filaments, applied themselves as closely as possible to flat surface. (Wigglesworth, 1937). Technically, this is an example of thigmotaxis as had been suggested from tissue culture studies by Loeb (1912), Loeb and Fleisher (1919), Harrison (1914) and other subsequent workers, and from experimental studies in regeneration of epithelium in animals, (Loeb, 1919-20; Arey, 1936; Wilhelm) and in insects, (Wigglesworth, 1936).

In/

In my experiments, the two spreading margins of cells covered the denuded surface of the mucosa within 72 hours by a single layer of flattened epithelium. The average distance between the two spreading margins was 2 - 3 mm. in each case. This rate of epithelialisation, in general, was of the order found in works of other investigators under comparable conditions of epithelial migration. Akaiwa (1919) found, experimentally, in 2 mm wide shallow skin wound in the ears of rats, 0.62 - 0.81 mm. distance was covered by spreading margin in 48 hours. Boling (1935) found a curettage, 3.2 mm. wide, of ciliated epithelium of nasal mucosa was resurfaced within 72 hours. Condon found that 1.5 - 2 mm. wide excised area of mucosa of trachea in rats was epithelialised by 72 hours. Wilhelm observed that a 2 mm. defect of curetted tracheal epithelium in rats was covered within 48 hours.

SUMMARY
(Tracheal epithelium)

By the method of direct injury, regeneration of the tracheal epithelium has been studied in normal rats. From such studies it has been observed that :

(1)/

(1) Following curettage, regeneration of tracheal epithelium begins with the migration of flattened cells derived from the marginal epithelium and exposed necks of submucosal glands; and the denuded surface (2 - 3 mm. wide) is covered by a single layer of flattened cells in 72 hours;

(2) In 3 - 4 days flattened cells become of simple stratified type;

(3) Differentiation begins on the fourth day and is complete in 33 - 45 days with complete functional recovery of the tracheal epithelium of the curetted area.

(4) Histochemical study of the tracheal epithelium is limited by the positive findings in the normal.

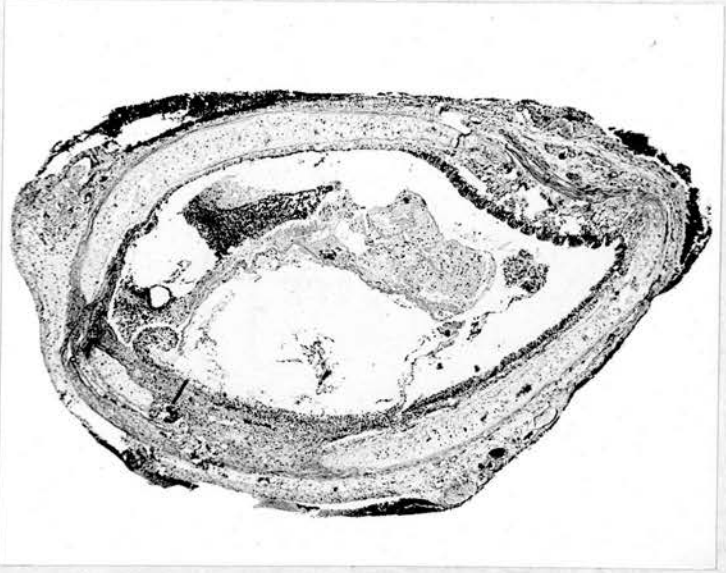

Fig.55 : 4 hours after curettage. Haemorrhage into the injured area and formation of fibrin upon the surface. x 30.

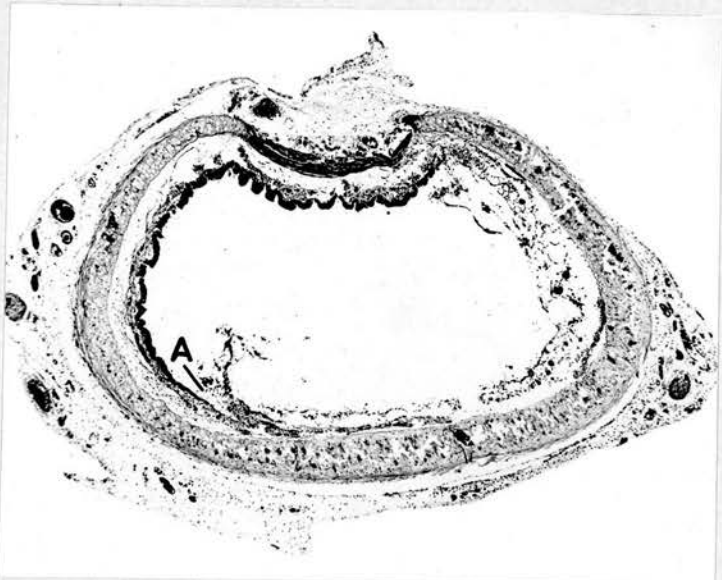

Fig.56 : 4 hours after curettage. Early marginal epithelial migration at (A). x 30.

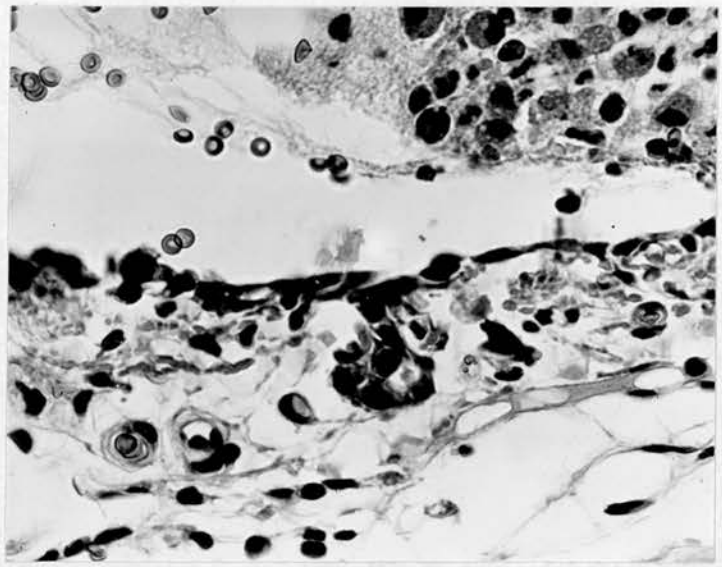

Fig.57 : 4 hours after curettage. Activity of basal cells extending from normal epithelium. (High power photograph of field marked A in Fig.56). x 575.

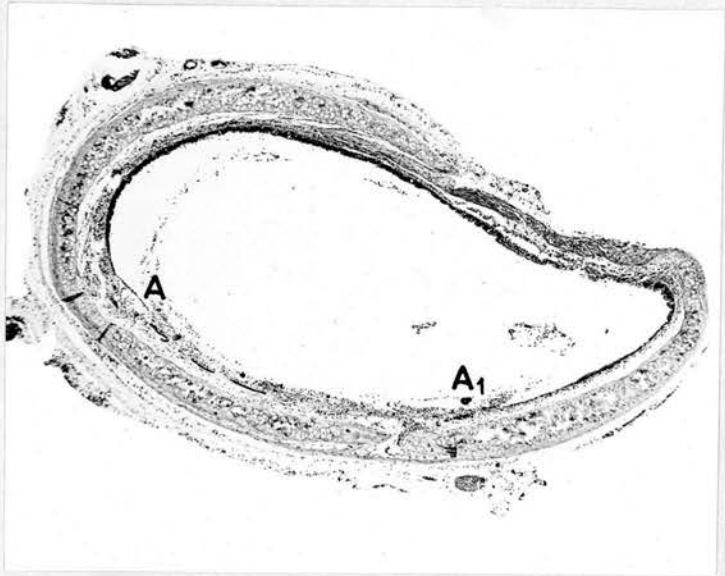

Fig.58 : 6 hours after curettage. Early marginal epithelial migration from both ends of the injured surface (A, A_1). x 30.

Fig.59 : 6 hours after curettage. Early migration of flattened epithelium. The extreme marginal ones are still ciliated. x 575.

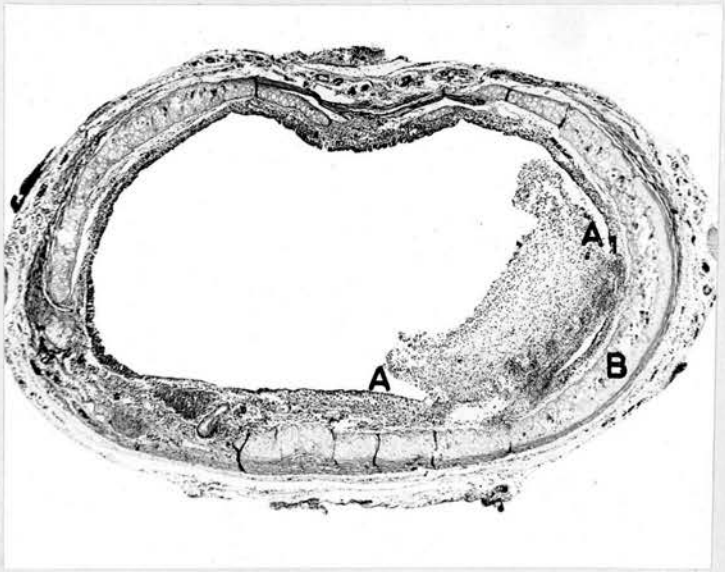

Fig.60 : 24 hours after curettage. Regenerating marginal cells are invading from both ends beneath the fibrin clot. Narrow clear zones are seen around and immediately ahead of the spreading cells (A, A_1). At B, extremely flattened cells derived from submucosal glands are seen under the fibrin clot. x 30.

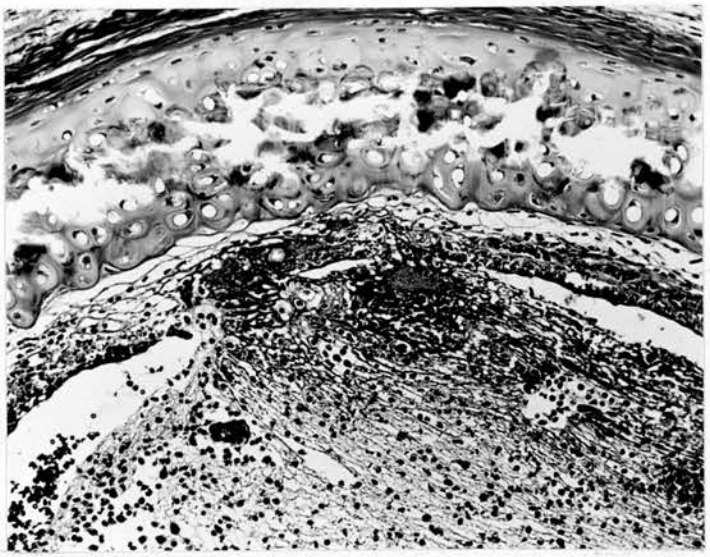

Fig.61 : 24 hours after curettage. The invading cells, 2 to 3 layers deep, from both ends almost casting off the fibrin coagulum. A line of epithelium of extremely flattened cells is seen under the fibrin clot. x 140.

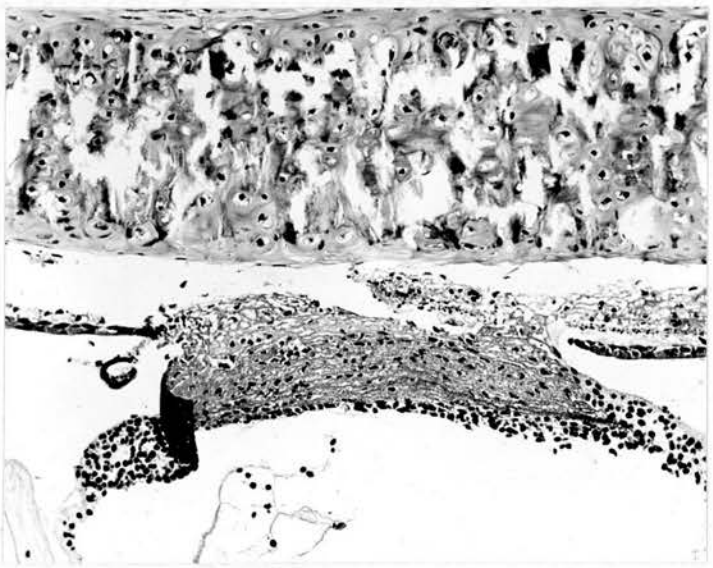

Fig.62 : 24 hours after curettage. The spreading margins are many layers deep with deeply staining cells. x 140.

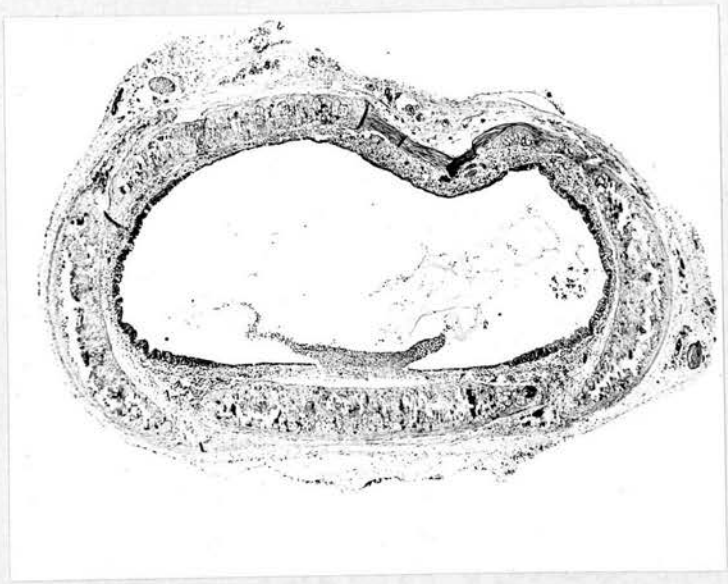

Fig.63 : 24 hours after curettage. About $\frac{2}{3}$ of the denuded area was covered up by the growing epithelium from the margin. x 30.

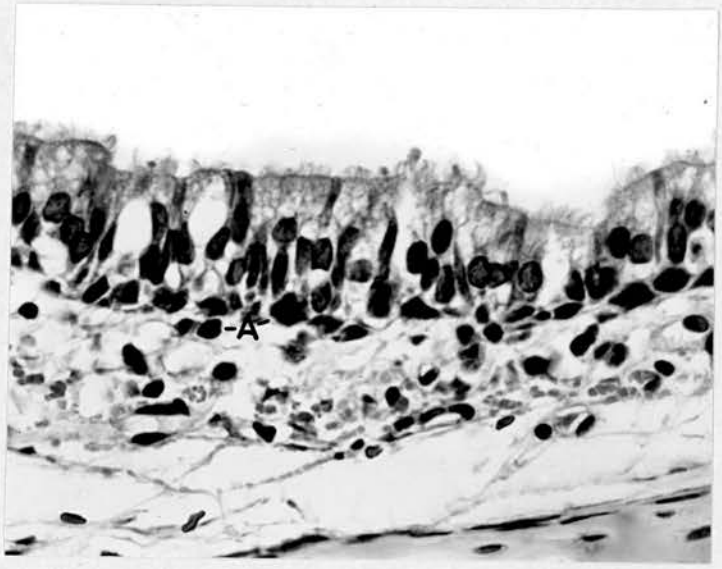

Fig.64 : 24 hours after curettage. Mitotic figures in uninjured part of epithelium beyond the margin of the migrating epithelium.(A). x 575.

Fig.65 : 48 hours after curettage. The whole surface was covered up by epithelial lining. x 30.

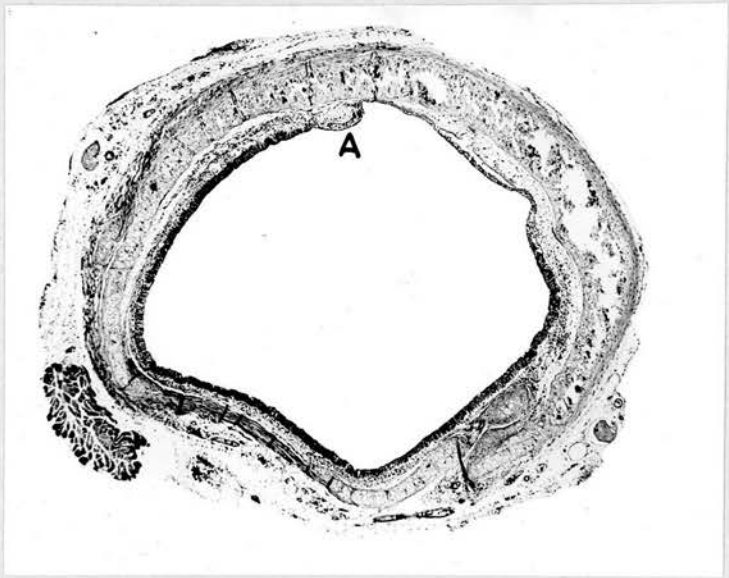

Fig.66 : By 72 hours after curettage epithelial covering of the denuded surface is complete. x 30.

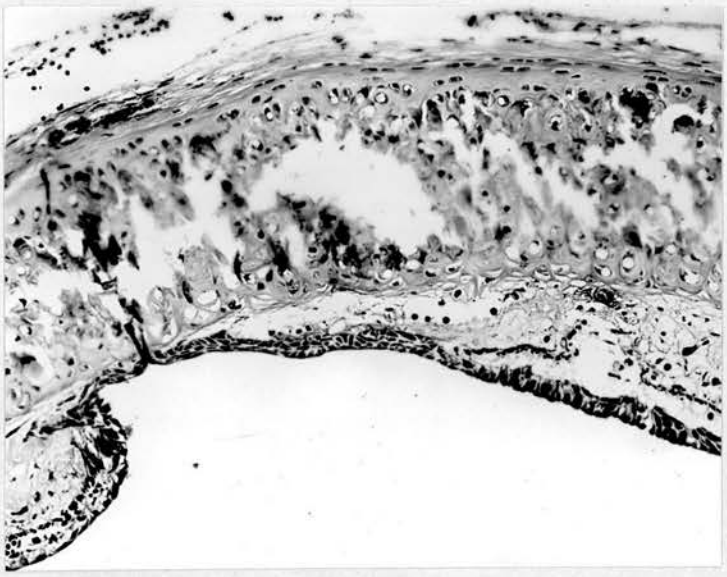

Fig.67 : High power view of Fig.66, at A, where stratified epithelium has covered the area of deep injury. x 140.

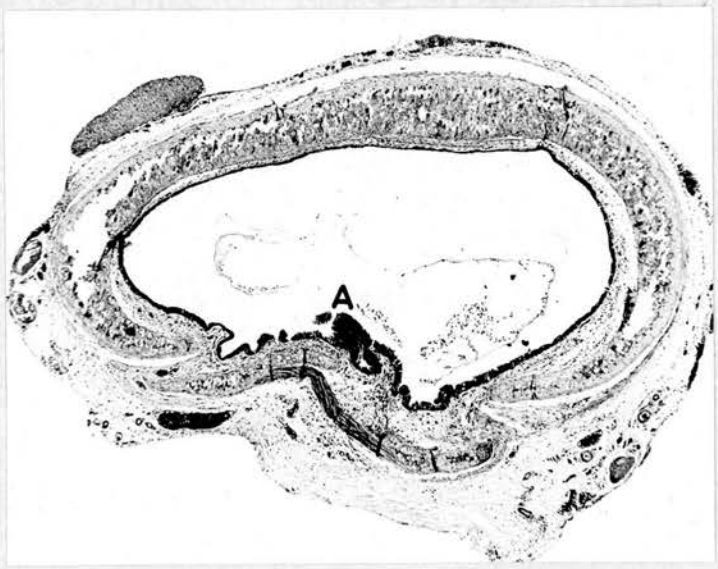

Fig.68 : 96 hours after curettage. Epithelial regeneration of the mucosa is complete. At A hyperplasia of the tracheal epithelium is seen. x 30.

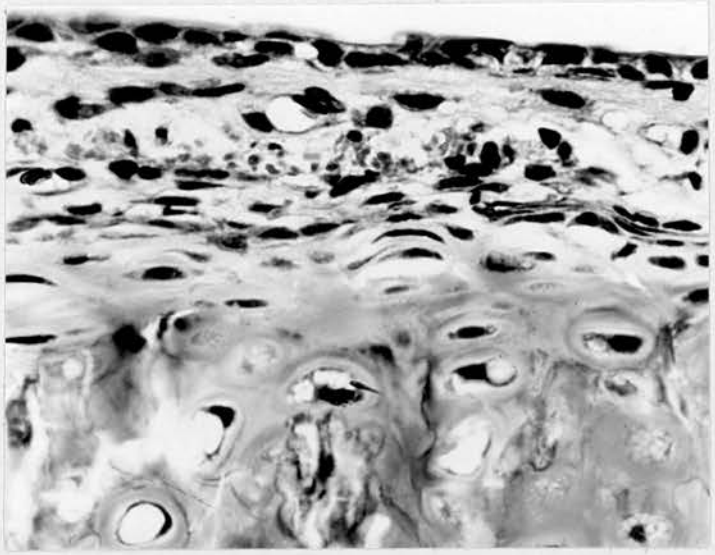

Fig.69 : 96 hours after curettage. Early formation of ciliated cuboidal cells. Minute masses of cilia are seen in the top layer. x 575.

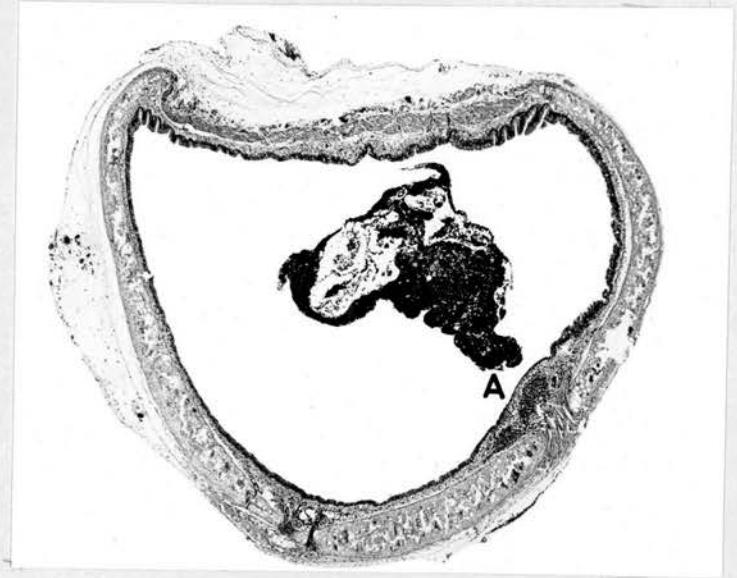

Fig.70 : 5 days after curettage. The denuded area is re-surfaced by cuboidal ciliated cells except in a small area at A. Cast-off fibrin coagulum is seen in the lumen. x 30.

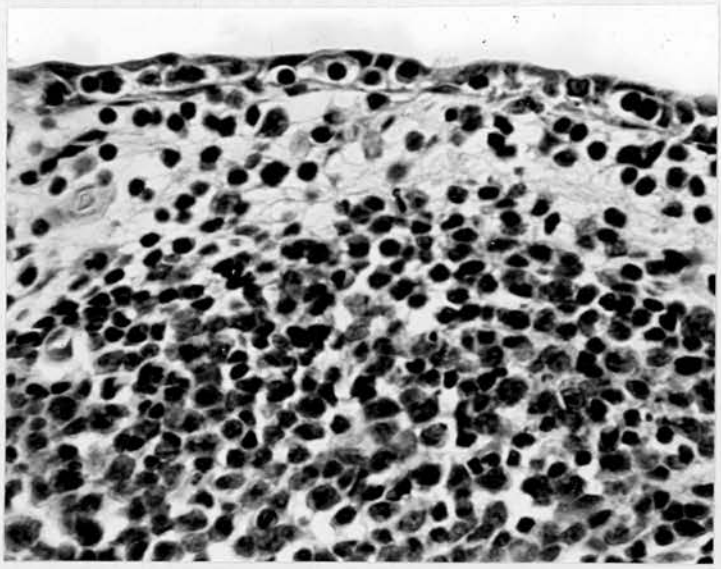

Fig.71 : 5 days after curettage. Regenerating tracheal epithelium with mitoses overlying an acutely inflammed mucosa. x 550.

Fig.72 : 5 days after curettage. Regeneration and orientation of the elastica of the mucosa. x 30.

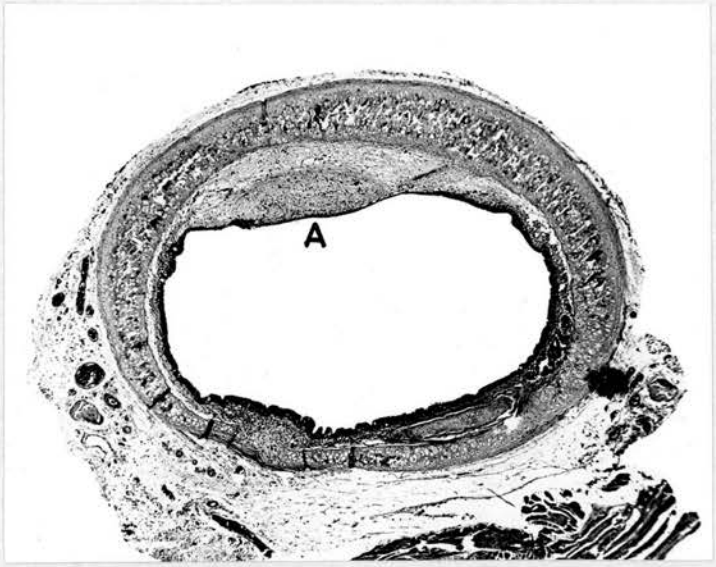

Fig.73 : 15 days after curettage. Except the deeply injured area(A) the whole surface was covered up by the redifferentiated epithelium. x 30.

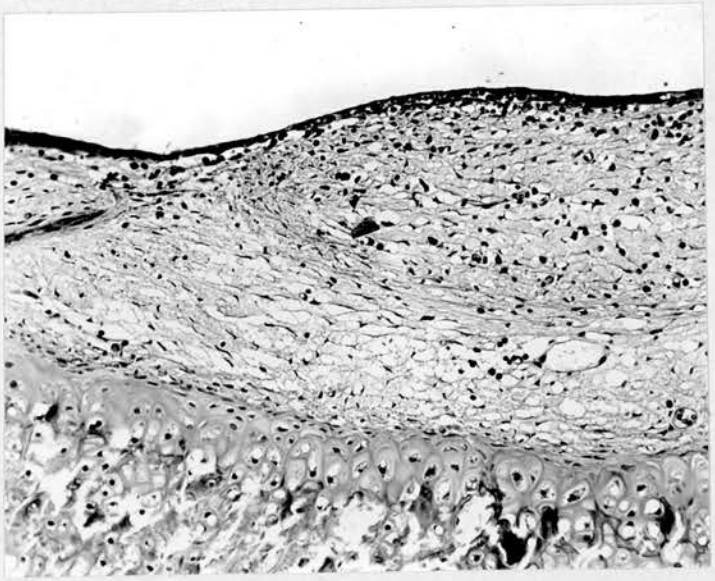

Fig.74 : High power view of Fig.73, at A, showing the epithelium in a stratified form. x 140.

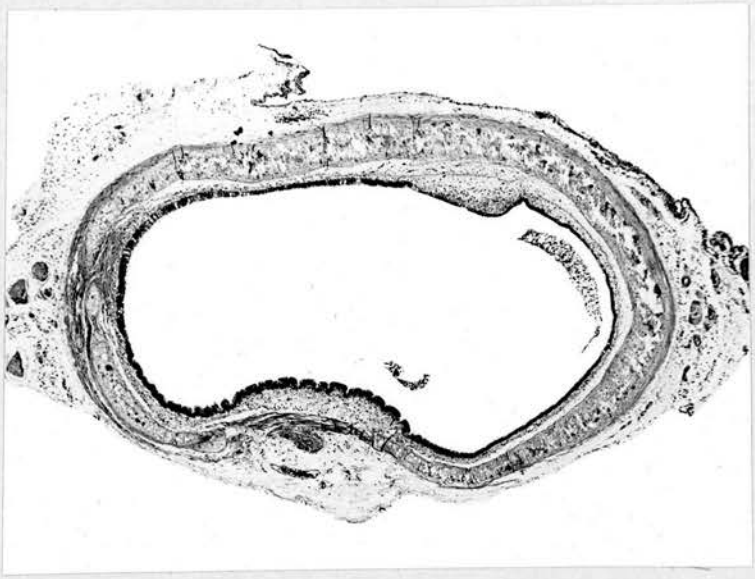

Fig.75 : 21 days after curettage. Normal epithelium covering the whole mucosal surface. x 30.

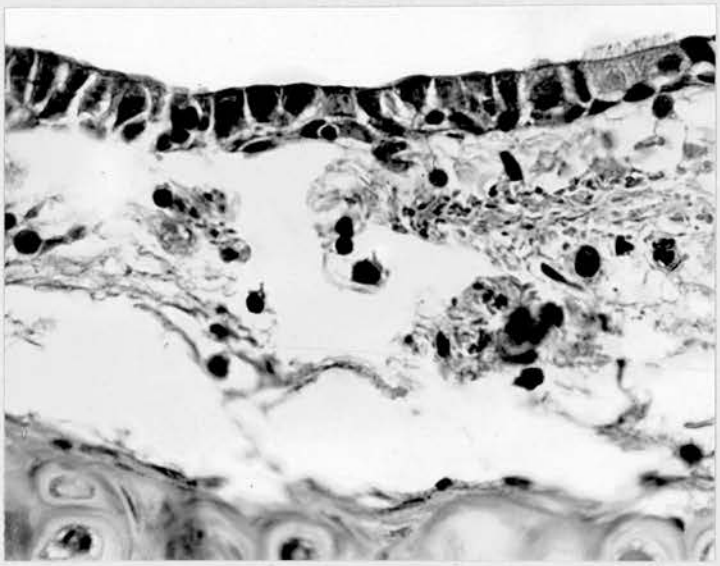

Fig.76 : High power of Fig.75, to show regenerated epithelium and goblet cells. x 550.

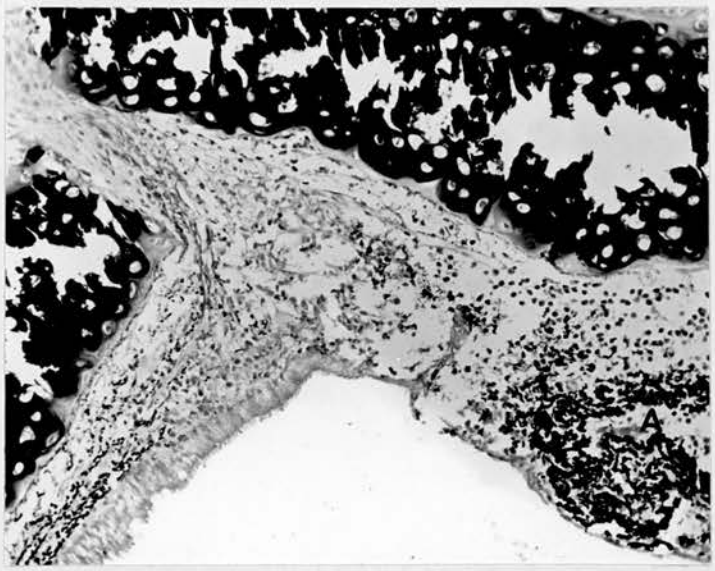

Fig.77 : 21 days after curettage. Proliferation of elastica at A in a deeply injured part of the mucosa. x 140.

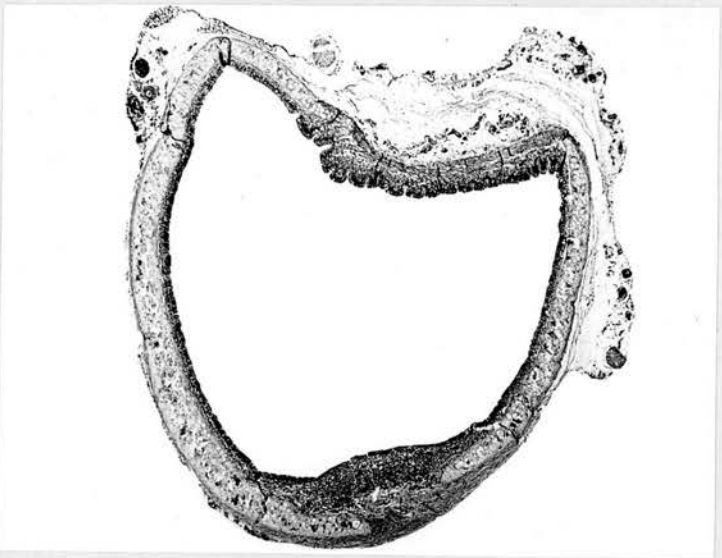

Fig.78 : 33 days after curettage. Ciliated low columnar cells covering the mucosal surface of the area of injury. x 30.

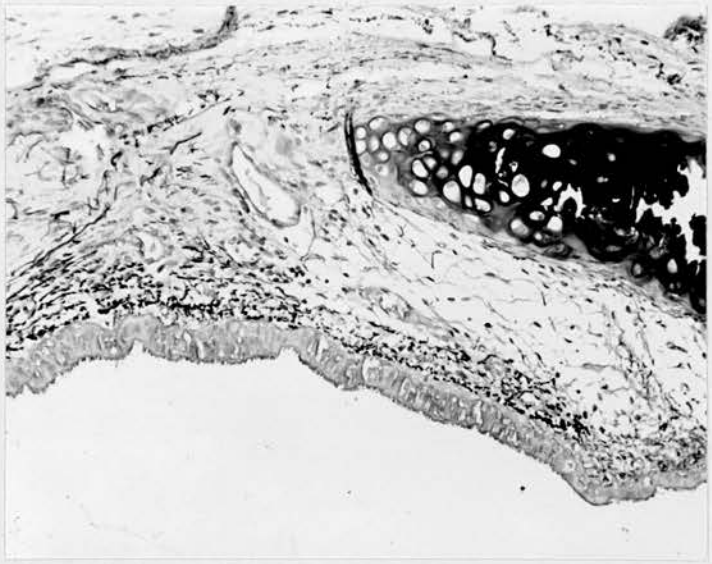

Fig.79 : 33 days after curettage. Regeneration and orientation of elastica in a damaged area of the lamina propria. x 140.

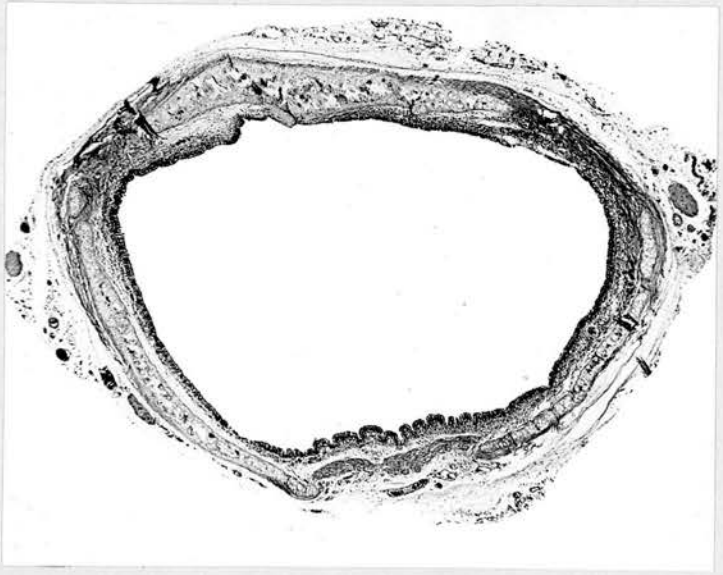

Fig.80 : 33 days after curettage. Epithelial lining is complete. x 30.

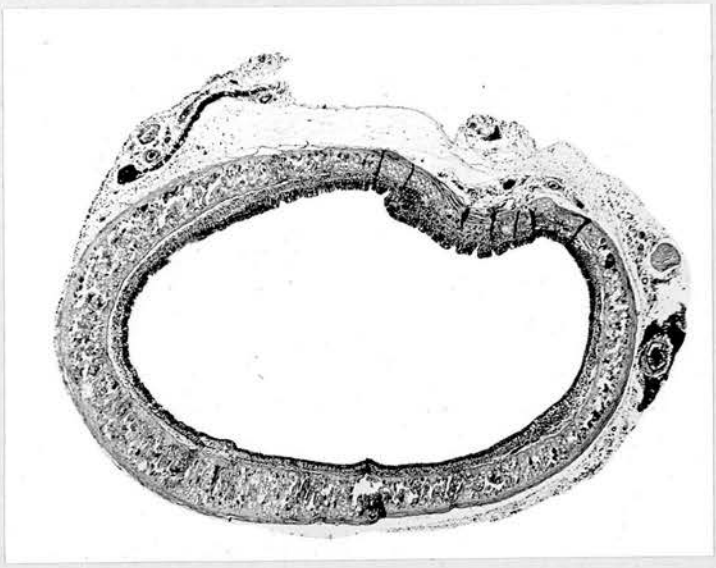

Fig.81 : 45 days after curettage. Complete regeneration of the mucosa with typical tracheal epithelium. x 30.

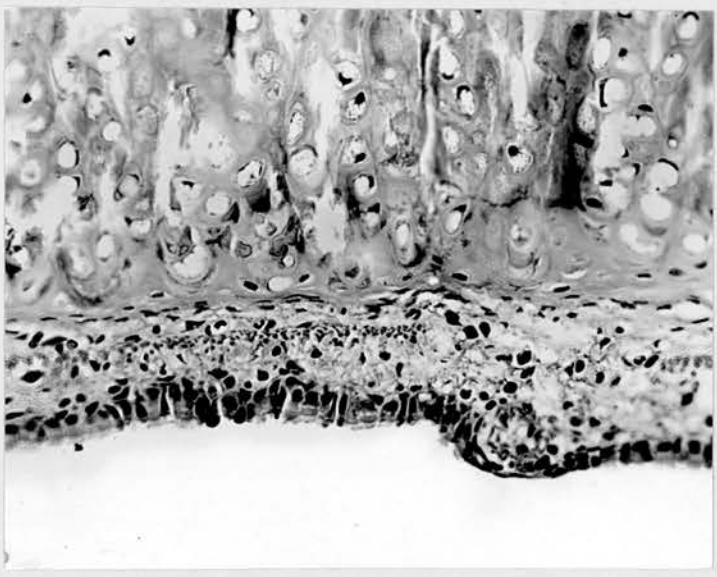

Fig.82 : 45 days after curettage. Ciliated columnar cells, from a regenerated area of epithelium of above figure in high power. x 275.

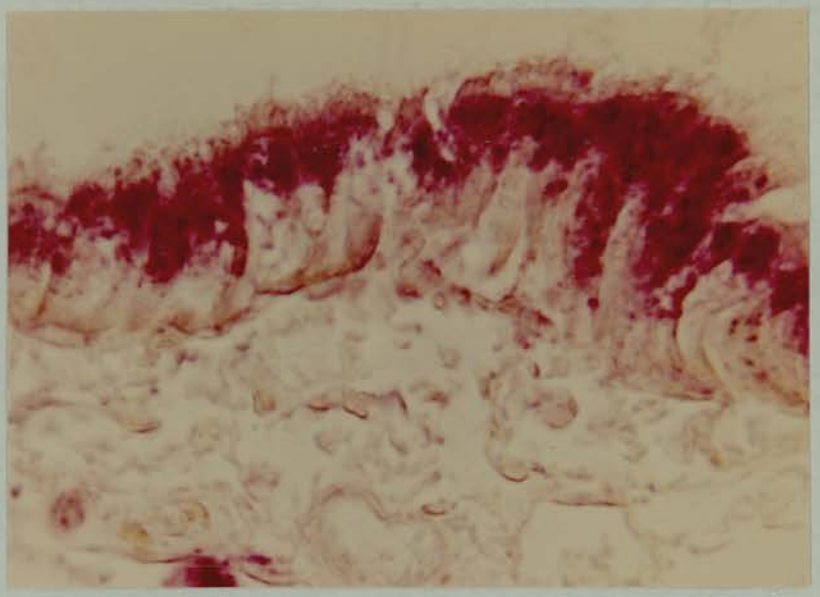

Fig.84 (Rat) : Normal trachea showing esterase reaction in the lining epithelium. x 1000.

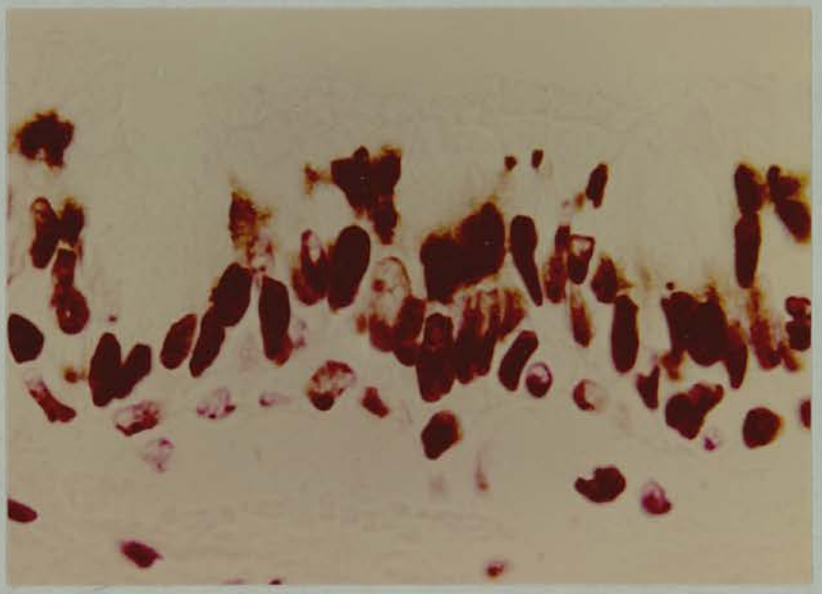

Fig.85 (Rat) : Normal trachea showing acid phosphatase reaction in the epithelial lining cells of the mucosa. x 1000.

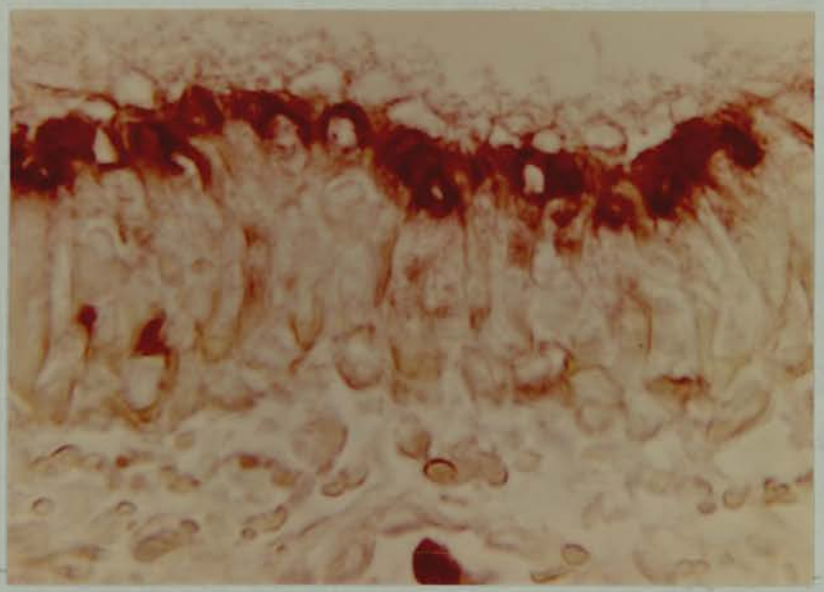

Fig.86 (Rat) : Normal trachea showing alkaline phosphatase reaction in the lining epithelium. x 1000.

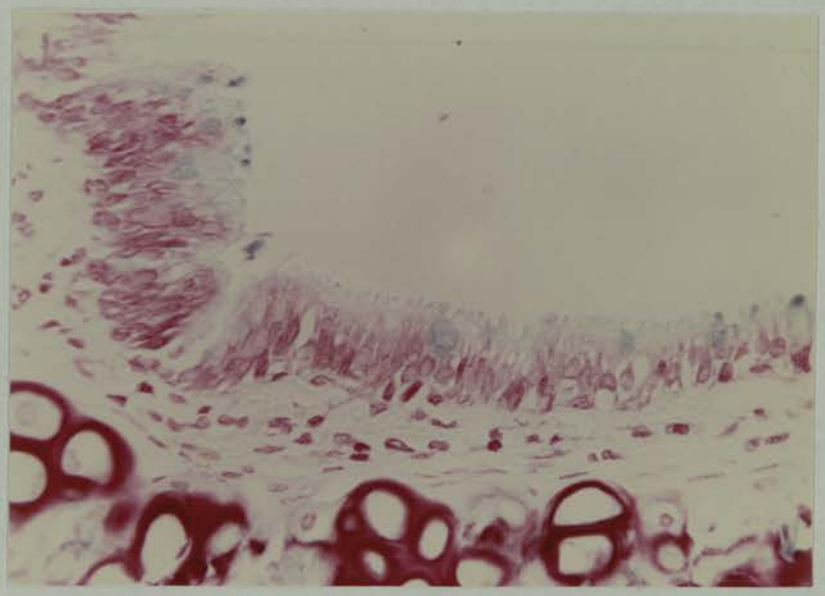

Fig.87 (Rat) : Normal trachea showing positive alcian blue reaction in the goblet cells of the mucosa. x 1000.

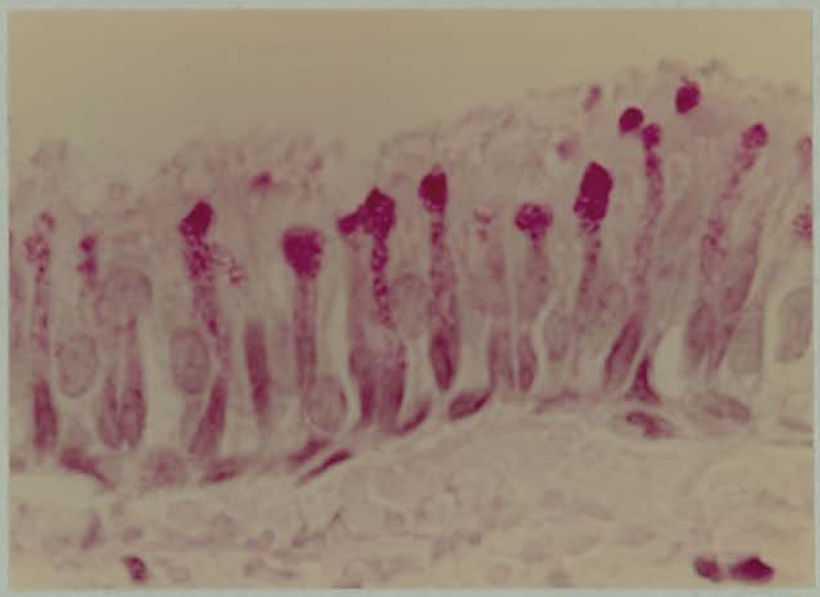

Fig.88 (Rat) : Normal trachea showing positive P.A.S. reaction in the goblet cells of the mucosa. x 700.

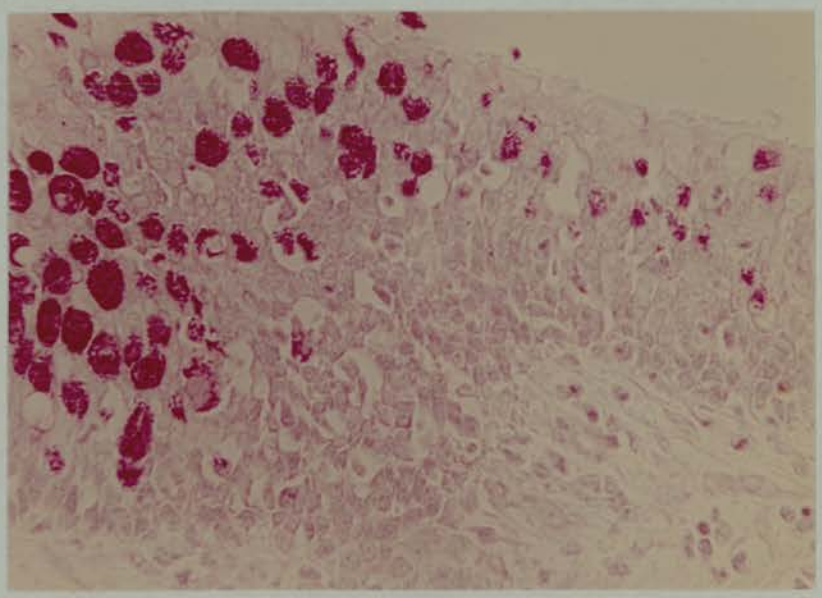

Fig.89 (Rat) : Normal tracheal mucosa showing strong activities for R.N.A. & D.N.A. x 700.

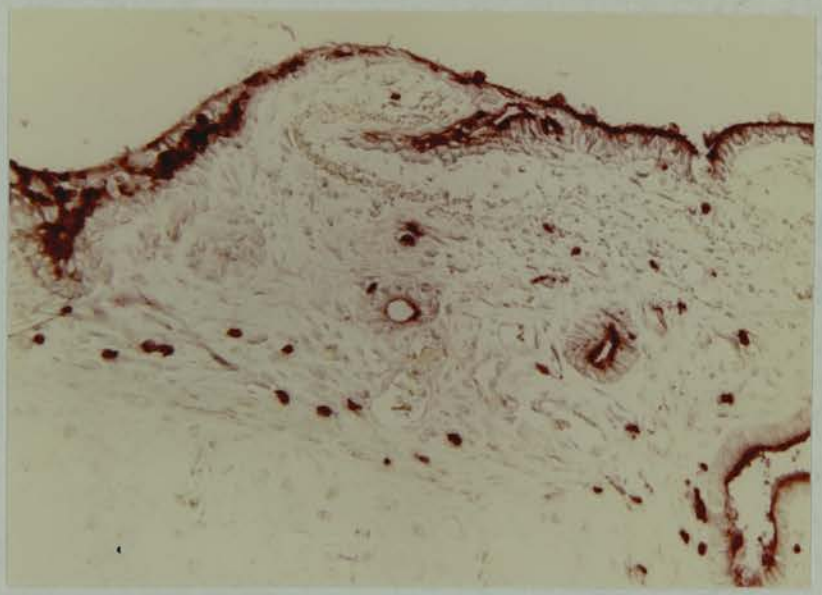

Fig.90 (Rat) : 72 hours after curettage. Acid phosphatase reaction in the flat epithelial cells of the regenerating epithelium. x 165.

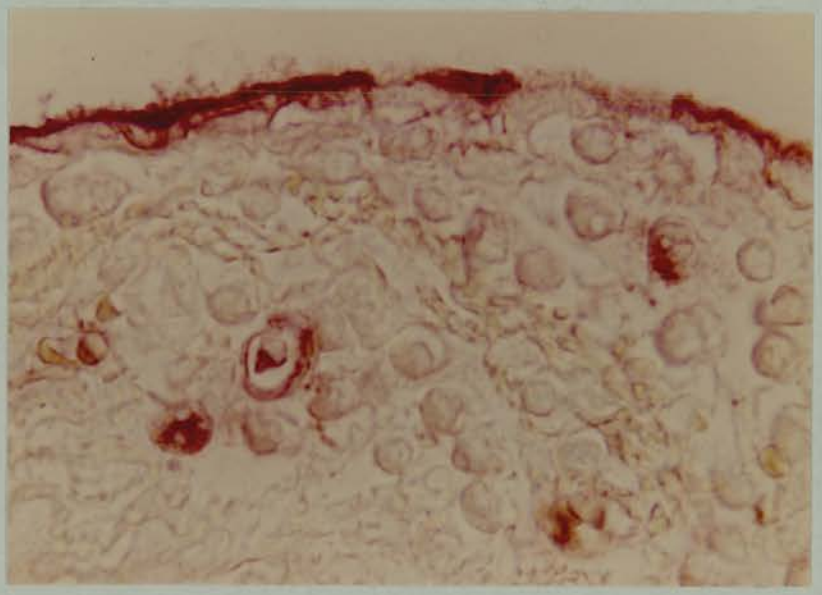

Fig.91 (Rat) : 5 days after curettage. Alkaline phosphatase reaction in the regenerating epithelium of the trachea, weaker than normal control. x 1000.

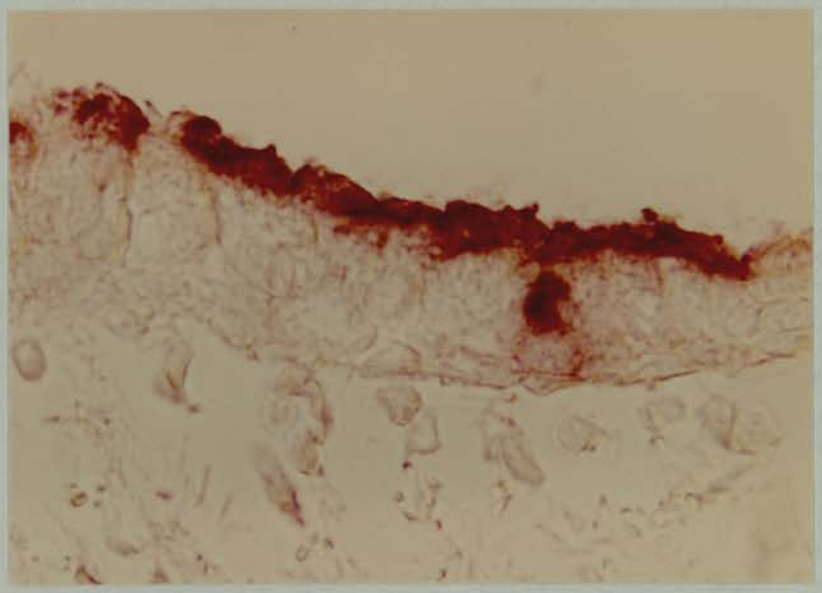

Fig.92 (Rat) : 2 weeks after curettage. Fairly strong alkaline phosphatase reaction in the regenerated epithelium of the trachea. x 1000.

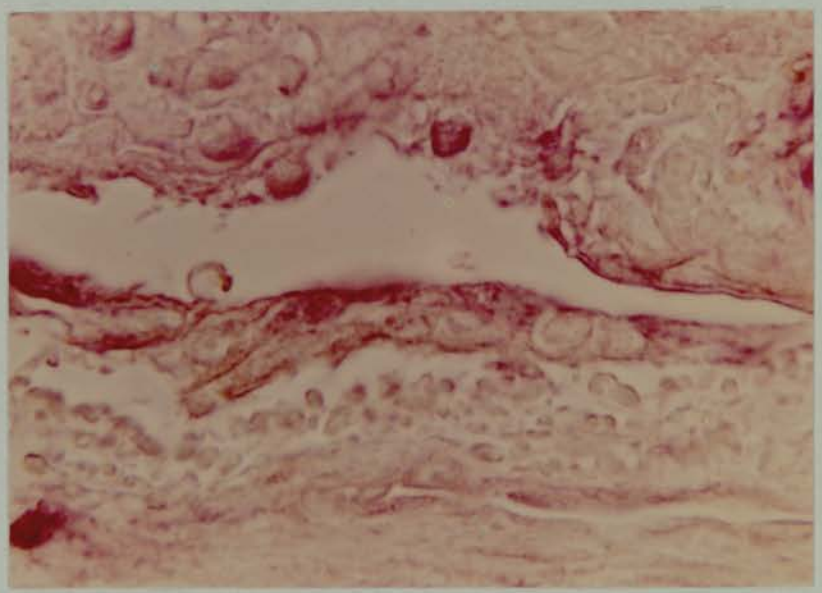

Fig.93 (Rat) : 6 hours after curettage. The flat migrating cells of the regenerating epithelium of the trachea show very mild esterase reaction. x 1000.

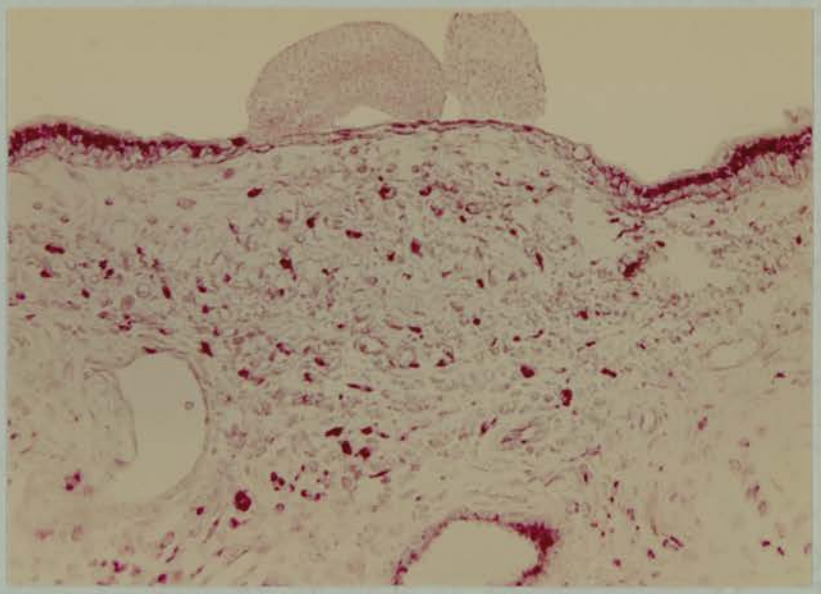

Fig.94 (Rat) : 2 weeks after curettage. Fairly strong esterase reaction in the regenerated epithelium of the trachea. The central part of the mucosa is poorly stained. The sub-mucosa, underneath the part, contains inflammatory cells. x 165.

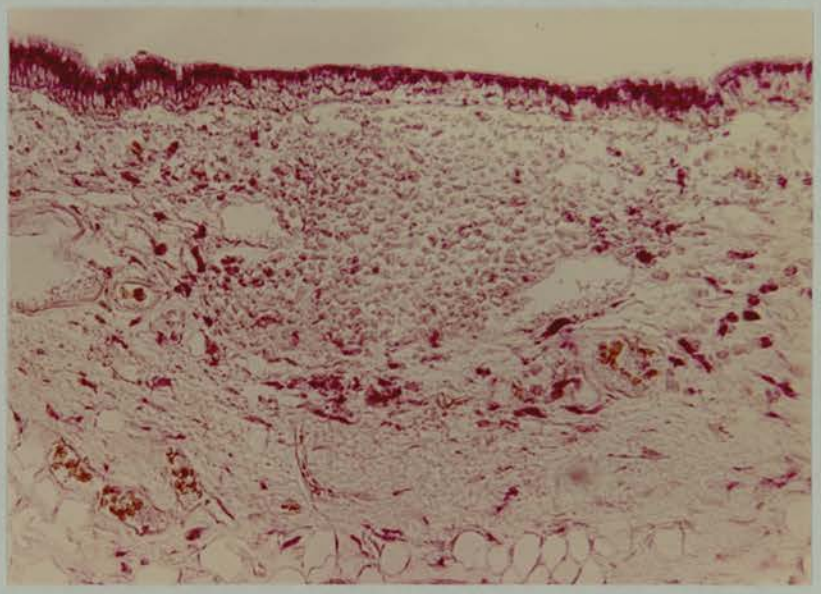

Fig.95 (Rat) : 45 days after curettage. Esterase reaction in fully differentiated epithelium showing almost normal intensity. x 165.

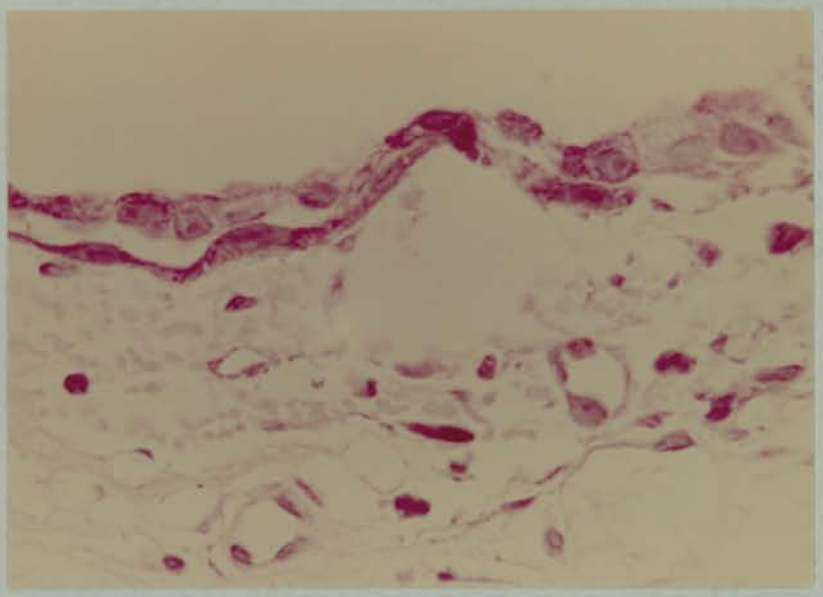

Fig.96 (Rat) : 6 hours after curettage. The flat migrating cells of the regenerating tracheal mucosa show very mild reactions for R.N.A. & D.N.A. x 700.

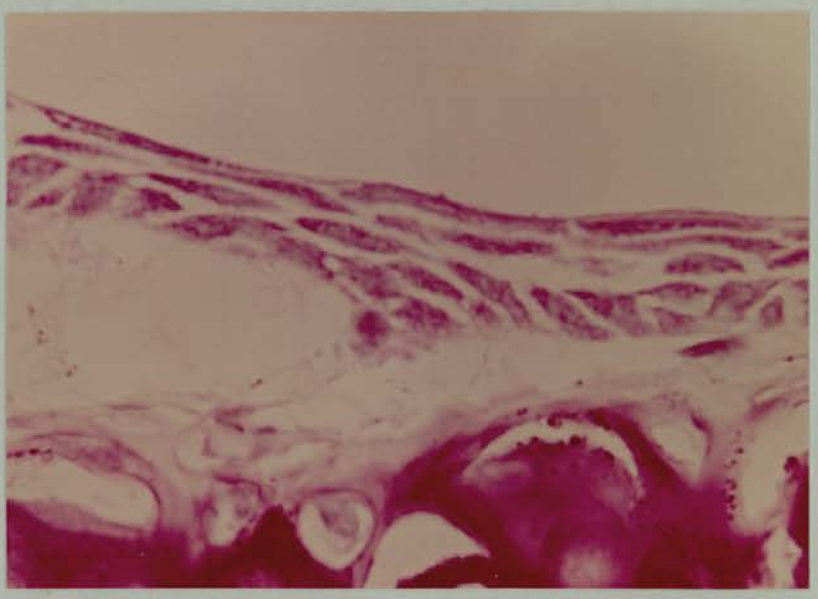

Fig.97 (Rat) : 24 hours after curettage. The many layered flat epithelial cells of the regenerating tracheal mucosa showing mild activities for R.N.A. & D.N.A. x 1000.

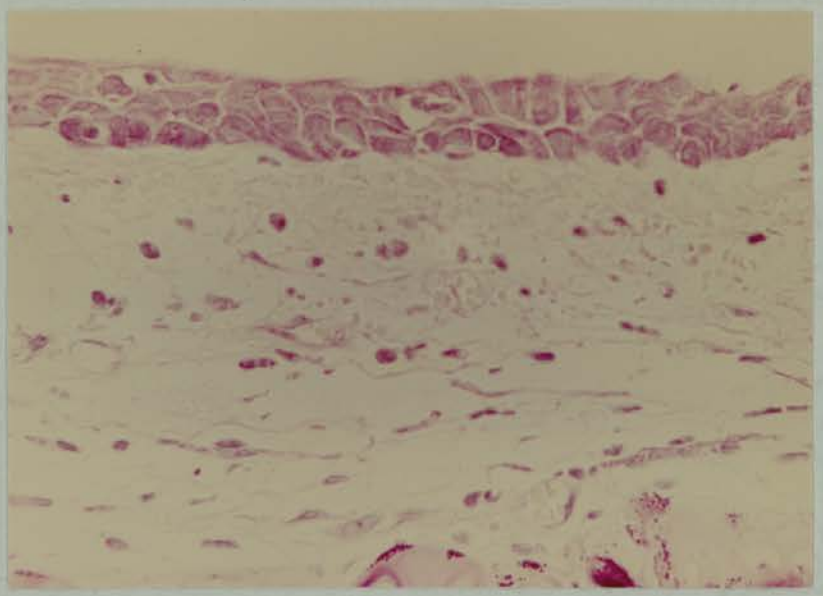

Fig.98 (Rat) : 7 days after curettage. The regenerated epithelium of the tracheal mucosa gives weak staining reactions for R.N.A. & D.N.A. x 400.

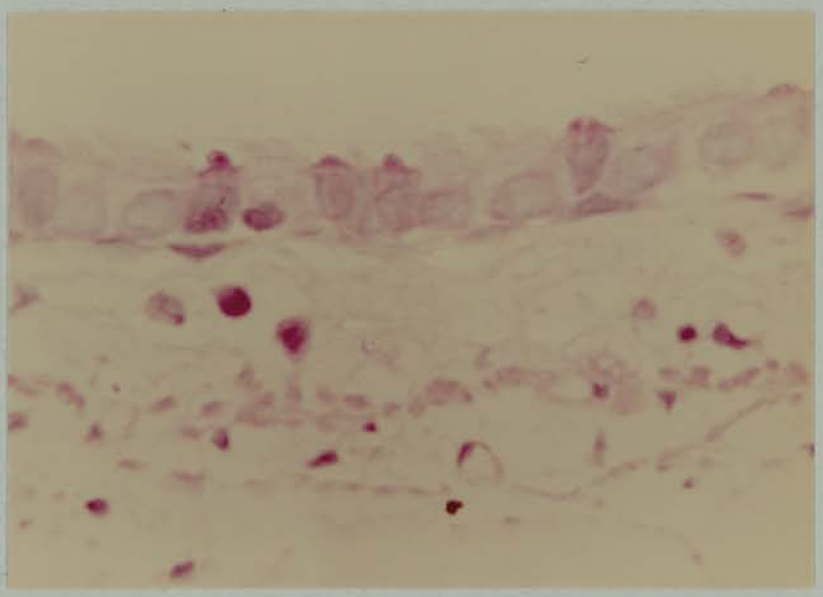

Fig.99 (Rat) : 3 weeks after curettage. The R.N.A. & D.N.A. reactions, in the regenerated epithelium of the trachea, are still weaker than normal control. x 1000.

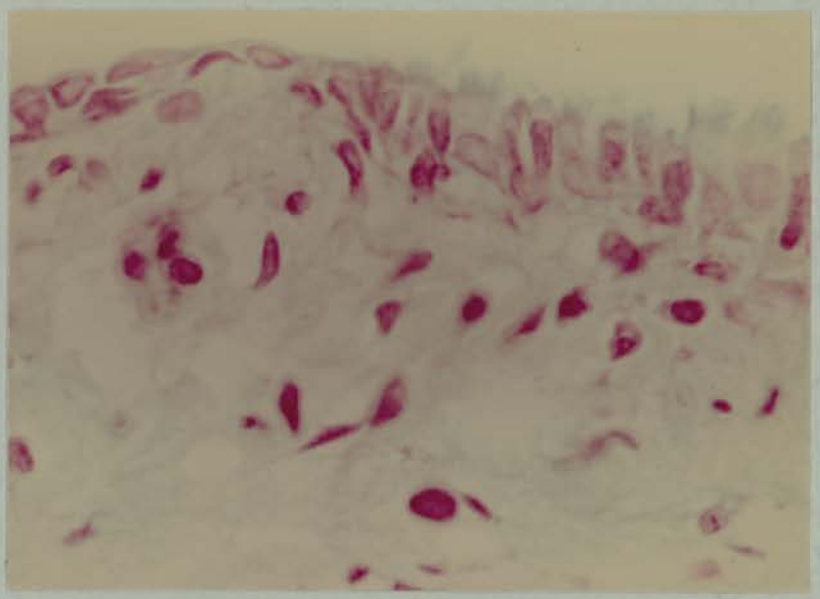

Fig.100 (Rat) : 45 days after curettage. Some of the mucous-secreting cells of the regenerated tracheal epithelium show positive alcian blue reaction in their free margin. x 700.

PART III

HEALING OF EXPERIMENTAL WOUNDS OF LUNG

SECTION I : STUDIES ON THE HEALING OF EXPERIMENTAL WOUNDS OF LUNG IN CATS.

SECTION II : AN AUTORADIOGRAPHIC STUDY OF THE UPTAKE BY THE PULMONARY EPITHELIUM OF $Na_2{}^{35}SO_4$ IN EXPERIMENTAL WOUNDS OF LUNG IN CATS.

SECTION I

(PART III)

STUDIES OF THE HEALING OF EXPERIMENTAL WOUNDS OF LUNG IN CATS

STUDIES ON THE HEALING OF EXPERIMENTAL WOUNDS OF LUNG IN CATS

INTRODUCTION

Lobectomy and pneumonectomy are, nowadays, very common surgical methods for removal of the damaged part of lung in tuberculosis, bronchiectasis, etc. The wound, thus produced by surgery, heals satisfactorily. But the process of healing of the wound is not clearly known in human subjects. A search of literature on experimental pathology on the study of the process of healing of wound of lung in animal has yielded two relevant papers of Olch and Ballon and of Montgomery as have been referred to previously in the introductory chapter.

This part of my thesis records experimental works designed to study the process of healing of lung-wounds in cat and to observe the degree of regeneration of lung tissue with the factors that govern the process. In particular, attention has been directed to the cytology of the process of healing with special reference to regeneration by the use of some histochemical methods to correlate repair of lung with that which is known as the histochemistry of repair in/

in the tissues.

EXPERIMENTAL PROCEDURE

20 healthy adult cats of both sexes were used in this series of experiment to study the pathology of healing of wounds of lung. The animals were usually kept under observation in the animal house, with proper care, for about a week prior to operation to avoid, specially, bronchopneumonia of cats from outside contamination. They were kept on usual normal cat diet, e.g., fish, milk, etc., during the whole period of experiment. Cat was taken as the animal of choice in these experimental works because cat's lung was particularly suitable for this type of work, and the results approximated more closely to those of human subjects than would be the case if rodents were used.

The animals were anaesthetised by intratracheal oxygen and ether under positive-pressure anaesthesia, pressure being kept steady at 75 mm. of mercury during operation. About a two-inches long skin incision was made between the 6th and 7th ribs on the left side and thoracotomy was performed by incising the muscle layers between these two ribs. The ribs were next retracted apart/

apart by a small self-retractor to get a full exposure of the interior of the chest. The upper part of the left lower lobe was exposed. The lower margin of this lobe was carefully withdrawn from the thorax on to gauze wrung out of sterile hot saline, which was used also to pack the edge of thethoracotomy wound and prevent the lung from slipping back into the chest. Two deep sutures of silk, one at the periphery and the other about a centimetre away from the first, were applied through the portion of lung to be removed as shown in Fig. 104, but not tied. With a sharp Mayo scissor a triangular wedge of lung tissue (each side approximately 2½ cm. long), from between the silk sutures was removed. Immediately after removal of the wedge of the lung the sutures were tied firmly to control bleeding from the severed pulmonary vessels of the cut surfaces. In most instances, there was considerable haemorrhage from one or more large vessels, and when a bronchus was involved, the blood frothed from mixture with air. As a rule the bleeding was controlled by tying the sutures firmly as above which also apposed the two cut surfaces of the lung/

lung. After this, the intrathoracic pressure was increased to about 100 - 125 mm. of mercury to see if there was any bleeding or any bronchial leak, and the movement of the lung was observed. Except in 3 cases there was neither bleeding nor hissing sound due to leakage of air from cut end of bronchus. In these 3 cases one more silk suture had to be applied firmly through the lung which was enough to stop the bleeding and leakage of air. Bleeding from the external wound was quite negligible. The movement of the lung was observed to be regular in all the cases. In order that the tissue might not be crushed, clamping and ligation of vessels were avoided as far as possible; clamps for the wound margins were never employed. Thereafter, the visceral pleural surfaces were carefully approximated by interrupted silk stitches, the lung restored to the chest, and the chest wall closed in layers by continuous sutures of No. 30 cotton after the pleural sac had been cleared of any fragments of blood-clot. As a rule the operative procedure occupied 30 - 35 minutes. Strict asepsis was observed throughout the experiment to avoid infection. The positive pressure was next reduced to/

to zero to allow the cat to breath by herself. At first the breathing was abdominal, and after a while it became thoraco-abdominal. The intra-tracheal canula was then removed. Penicillin (200,000 units) and streptomycin (½ g.) combined were injected into each animal daily for 3 days as an anti-infective measure.

The animals were killed at intervals of from 24 hours - 130 days after operation. Six cats were killed on alternate days in the first week of operation, and another 4 in the first month at various intervals. Subsequently, 3 cats were sacrificed during the 2nd and 3rd months, and 3, four months after operation. Unfortunately, 2 animals died during operation and another 2 from severe infection of the wounds, both external and internal, within a few days of operation. The animals were usually killed by intra-peritoneal injection of nembutal (10 c.c.).

After death, the external wound was opened to see ifthere was any infection. The chest was opened next through the midline and the heart and the lungs were taken out en masse. Small blocks of lung tissue were cut from the areas of the wounds and fixed in suitable fixa-

tives/

tives for different staining methods. The lungs were usually not expanded with intra-tracheal formol-saline, except one or two cases, as the formol-saline might damage the chemical integrity of cells which was observed to be harmful for the correct result of the histo-chemistry of tissue. The other parts of both lungs were examined and were found normal.

The blocks of tissues were fixed in 10 per cent formol-saline for 48 hours for haemo-toxylin and eosin, Hart's elastic tissue stain, picro-Mallory for connective tissue stain, P.A.S. and alcian blue reactions; in cold acetone (4°C) for 48 hours for enzyme reactions and in modified Baker's fluid for R.N.A. and D.N.A. These were next dehydrated and embedded in paraffin wax in vaccuo. Thick and thin (5u) sections were stained by Ehrlich's haemotoxylin and eosin, modified picro-Mallory and by Hart's elastic tissue methods. For histochemical examination, suitable sections were stained by alcian blue and P.A.S. methods for mucopoly-saccharide, Kurnick's plasma-cell stain for R.N.A. and D.N.A. and for enzyme reactions the sections were stained by modified Gomori's histochemical methods.

OBSERVATIONS/

OBSERVATIONS AND RESULTS

Recovery of the animals from surgical procedure was rapid and they seemed little upset. The external wounds of the skin healed completely by a week or so, but it took longer if there was any infection. Skin infection did not always affect the interior of the chest. It was found, in two cats, that in spite of severe infection in the thoracotomy wound the lung and the pleura were not at all affected. On the other hand, it had been observed that there were firm adhesions of the pleura to the diaphragm and the chest wall in one case where the skin wound was perfectly healed.

On naked-eye examination of the lung, the wounds in animals killed within the first week, the scars were clearly defined as dark haemorrhagic bands protruding slightly above the pleural surface and surrounded by congested lung tissue, but later the result was a depressed scar varying in colour with age from dark purple to light blue. As a rule the scars within the first month were surrounded by a zone of whitish pleura with a limited pleural reaction.

To summarize, the wound of the lung inflicted/

ted by the excision of a wedge of lung tissue in cats, heals by scar formation leaving behind a dimple on the pleural surface, free from adhesions provided care is taken to appose the pleural surfaces reasonably accurately and to see that all bleeding is controlled before the lung is returned to the thorax with strict surgical asepsis.

Microscopical Observations

For the convenience of description, the whole range of repair of the wounds of lung has been divided into three different periods. The first period includes the wounds of cats, killed at intervals of from 24 - 96 hours; the second period covers the wounds of the first, second and third weeks and in the third, 5 - 18 week-old wounds were described.

24 - 96 hours after operation

Microscopical observation on the healing processes of the wounds of lung in the earliest phase of repair revealed a comparatively local and limited reaction to the tissue around the wound. The gap of the wound was occupied by an irregular mass of blood-clot (Figs. 105, 106/

106) surrounded by collapsed or partially collapsed alveoli. Strands of fibrin were present in the clot and extended over the pleural surface for a variable distance. Beyond the collapsed alveoli the lung tissue was congested and some smaller vessels were seen stuffed with blood. Appearance of inflammatory cells at this stage was less evident. There was necrosis of cells near the surface of the wound. Dead or dying cells, some with irregular swollen nuclei were found in the alveoli. Dilated blood vessels and collagen fibrils were seen in the sub-serous cellular zone but fibroblastic reaction was absent in the first 24 hours of repair. Many of the alveolar and subpleural capillaries were dilated and lined by swollen endothelium. In places there were few subpleural vascular fibroblastic reactions, derived from surface alveoli after 24 hours. Fig. 108 is a low power view of a 60 hours-old wound the central part of which was still occupied by a dark mass of blood-clot. The tissue reaction by this time was quite advanced locally and also in surrounding areas as evidenced by the proliferative activities of young fibroblast cells. (Figs. 111, 117). Fig. 107 and 109 show subpleural early/

early fibroblastic reaction.

Large round macrophage cells, which appeared to bud from alveolar walls (Figs. 119 & 140 - 11 days), specially in the region beneath the sub-serous cellular zone, were quite frequent. The alveoli which were not collapsed near the wound had swollen lining cells, cubical or polyhedral in shape. Strands of fibrin were present in the clot and extended over the pleural surface covering and replacing the serosal cells. Non-haemolysed individual red corpuscles were present in the gap of the wound as late as 96 hours after operation.

The alveolar cells were swollen and hyperplastic specially in the superficial zone. Many of these cells had assumed cubical or polyhedral shape and all had large vesicular nuclei. Macrophage cells were noted where blood was present and phagocytosis had been observed in these areas. (Fig. 111). Polymorphonuclear leukocytes were abundant in the reactive zone, more in the vicinity of the suture. The inflammatory reaction with cellular infiltration of mononuclear or lymphocytic type was seen at its peak in 96 hours after operation, with pus cells around the suture.

Newly/

Newly-formed collagen fibrils and spindle-shaped fibroblast cells were seen in abundance, by now, near the margins of the wound, 60 - 96 hours old, (Fig. 117) indicating the beginning of organisation of the haematoma of the wound gap. Many of the alveolar capillaries had dilated, packed with erythrocytes and were lined by swollen endothelium.

Pleural Reaction: In excising wedges of lung tissue, a corresponding portion of pleura was removed and, although edges were approximated as neatly as possible, between the sutures small areas of lung tissue were left denuded of their serosa; these were speedily covered by fibrin. (Fig. 109, top left). Subpleural alveolar endothelial cells, derived from the surface alveoli, and vascular fibroblastic reaction directed towards the re-formation of the pleural membrane was evident in 48 - 60 hours-old wound. So far as could be determined, the endothelium of the superficial alveolar capillaries became swollen and hyperplastic, many assuming cubical and polygonal shapes with large vesicular nuclei and prominent nucleoli, and took important part in the formation of collagen for ultimate restoration of the pleural membrane.

The/

The endothelium of the alveolar capillaries which had undergone the above changes was arranged irregularly in columns and sheets (Fig. 107) tending to form a delimiting barrier between the subserous cellular zone and the normal alveolar cells deeper in the lung at the margins of wound beneath the existing pleura. This reaction was further accentuated by similar reaction of the subpleural alveolar and capillary endothelium in the region of the interrupted pleura, on its pulmonary aspect, which was inverted for a varying distance into the wound. (Fig. 109). Simultaneously, with the subpleural reaction, there was cellular reaction superficial or external to the limiting elastic membrane of the lung. A vigorous serosal cell reaction took place at the periphery of the fibrin deposited on the pleural surface, where pleura for varying distances around the wound was largely denuded of serosal cells and covered by fibrin strands continuous with the superficial fibrin-clot of the wound itself, or beneath the fibrin wherever surviving serosal cells remained. The latter proliferated rapidly and grew over and into the fibrin in spindle-shaped forms (Fig. 110, 113), many of them morphologically indistinguishable/

indistinguishable from fibroblasts, which took part in the organisation of the surface fibrin. Subsequent stages of pleural reaction towards the re-formation of the pleural membrane varied only in intensity and was described in the following pages.

Regeneration of lung tissue: Evidence of regeneration of lung tissue was not seen within 48 hours of operation though it was probable that the process commenced immediately after the traumatic stimulus. Proliferative activity of bronchial epithelium near the wound, with the formation of new outgrowths from the parent stems, was the earliest sign of regeneration. As early as 60 hours after operation, the proliferative reaction of epithelium could be observed both in the large and small bronchi around the wound. Fig. 112, a low power photograph of a 96 hours-old wound of lung, shows the advanced stage of repair of the wound where the blood in the wound gap had been gradually replaced by organising tissues. Both in the central and peripheral part of the wound proliferative activity of bronchial epithelium gave rise to bronchial buds. In the larger bronchi the earliest sign of regeneration was the development/

ment of cubical cells which replaced the tall ciliated columnar epithelium at one or more points in the lumen. This was succeeded by their appearance external to the membrana propria in groups of proliferated cells (Figs. 114 & 115). These solid masses of epithelium formed bronchial buds which were characteristically in the form of narrow channels of cubical epithelium. (Figs. 126, 131, 132). This proliferative response on the part of large and small bronchi simultaneously, was seen to occur on the aspect of the bronchus directed towards the wound.

Mitotic activity was quite frequent in the proliferatingepithelium and in some wandering cells in the region of early organisation where large macrophage cells, actively phatocytic, lie side by side. Fig. 111, a high power photomicrograph of a 60 days-old wound, shows an area of early organisation with a mitotic figure in its metaphase and many macrophage cells. On the left hand side of this figure, a bronchus is seen with proliferating epithelium from which masses of epithelial cells have started to grow into the organising area. Figs. 118 and 120 show mitoses in similar areas of early/

early organisation with young fibroblast cells, capillaries and actively phagocytic macrophage cells. Frequency of mitotic activity was first observed to occur in the proliferated bronchial epithelium and bronchial buds near the wounds, 48 - 60 hours old, and continued to appear in all the stages of repair.

6 - 25 days after operation

Progressing from the fourth day through the first week, the inflammatory reaction gradually subsided except in the immediate vicinity of the sutures where pus cells were abundant. Macrophage cells were very active in removing the debris of the wound and remained such till the last stage of repair. The blood-clot in the gap of the wound was gradually replaced by young granulation tissue consisting of fibroblast cells and young capillaries (Figs. 116, 124). The progress of the repair is illustrated in Figs. 121, 129, 135 & 147 which represents 6, 8, 11, 12 and 20 days-old wounds respectively. In Fig. 121, a 6 days-old wound, the haematoma of the wound is seen almost completely replaced by organised tissue all round except a little subpleural zone on the right hand/

hand side. There was no haematoma left in the wounds of older age. Organisation proceeded from the periphery and subpleural region of the wound towards the centre till the entire haematoma was replaced. Numerous bronchial buds are seen sprouting out from the peripheral bronchi in all the illustrations and a considerable portion of the lung tissue is seen aerated in 20 days.

Pleural Reaction: The serosal cell reaction was very marked at this stage. The spindle-shaped cells grew under the fibrin-clot to help in the organisation of the surface fibrin which ultimately became separated from the new pleural membrane. (Fig. 160). Masses of collagen fibres were seen to form in the line of reformation of the new pleura, separating the pulmonary tissue from the overlying organised fibrin mass. (Fig. 123). This reaction together with the proliferative activity of the subpleural alveolar and capillary endothelium in an attempt at the re-constitution of the pleura, resulted in the formation of a dimpled but a continuous limiting membrane of the lung (Fig. 142) by the union of similar proliferative tissue from other angles of the surface of the wound/

wound under ideal conditions. This new tissue was a fibro-elastic membrane which thickened and in 12 days time was connected by loop-like processes with the elastic fibres of the re-expanding marginal alveoli. (Fig. 143). The tissue superficial or external to the new pleural membrane, derived also from serosal cell reaction, was gradually cast off (Fig. 122, 129, 130, 135) and the pleura though thickened at this stage, was seen limiting the wound like that of normal lung. (Figs. 142, 147, 152, 162, 163).

Regeneration of Lung tissue: With the ageing of the wound, the healed area of lung tissue gradually became aerated, mainly in three noticeable ways: (1) Bronchial budding and formation of new alveoli, (2) Expansion of collapsed alveoli, and, (3) The splitting of collagen into slit-like spaces. First in importance was the formation of bronchial buds from the proliferating epithelium of bronchi adjacent to the traumatized area. This was the major contribution towards the re-aeration of the lung. As the wound aged, the bronchial buds increased in number in so far as their wall came to contain muscle fibres and elastic tissue. The buds were first formed as solid masses of epithelial/

epithelial cells (Figs. 125, 132) which were subsequently canalised by the air pressure from the main stem. (Figs. 126, 131, 133). The canalised buds, in course of time, grew into the secondary and tertiary branches. From the terminal division new alveoli were formed. (Figs. 127, 128, 137, 141, 144, 148-150). These new alveoli were lined by cubical cells similar to that of new bronchial divisions. Alveoli were also found to grow directly from the original bud. The new alveolar spaces were usually filled up with debris and many macrophage cells were found in these spaces with active phagocytosis. (Fig. 134). The newly-formed bronchi from epithelial buds were lined by cubical cells which with the expansion of the bronchi reverted to a tall ciliated type on one hand, and on the other they were transformed into flattened, elongated epithelium to line the new alveoli. These changes were difficult to illustrate, though in some instances they were more or less detectable as seen in Figs. 149 and 157, where the new bronchi are seen partly lined by tall ciliated type of cells and in Figs. 158 and 159 (35 days) cubical cells are seen to transform into flattened epithelium. Examples of regenerated bronchi and/

and alveoli originated from epithelial buds are illustrated in Figs. 126, 127, 136, 140, 144.

Mitotic figures were seen in abundance in the proliferating bronchial epithelium, epithelial buds and in the lining cells of the new alveoli. (Figs. 126, 132, 133, 137-141). These nuclear figures were not infrequent even in the late stages of repair of the wound. (Figs. 149, 150).

Simultaneously, with this major process of regeneration, two other subsidiary developments occurred in the older wounds as further contribution to the aeration of the lung. They were, as mentioned above, re-expansion of collapsed alveoli and formation of new air spaces in the fibrous areas of the lung.

Many of the collapsed alveoli, which were found borderingthe immediately traumatized area and the blood-clot of the wound together with others whose definition was lost and whose capillaries had dilated to form a vascular barrier to the wound, were found to expand as the wound healed. The appearances of these expanded alveoli in a wound of several days' duration was of columns of alveolar cells arranged more or less parallel to one another, bordering linear/

linear air spaces and slanting towards the wound at angles which diminished from the surface to the apex. (Fig. 151).

The aeration of collagen tissue in the central or apical portions of the scar of the wound took place by splitting of the tissue. Irregular slits developed in the collagen. They were usually lined by cubical cells but often their lining cells might be elongated, flattened and spindle-shaped (Fig. 146). These slits were always in connection with bronchial buds or alveoli. Sometimes they appeared to communicate with blood vessels and contained red blood corpuscles. The alveoli which bordered or formed in the margins of the scar were lined by cubical cells in part or in whole; if the former, it was the aspect in contact with the denser tissue which was cubical (Fig. 136). Sometimes such alveoli, wholly lined by cubical cells, were found in the central part of dense collagenous tissue. (Fig. 145). In other instances, groups of alveoli lined by cubical epithelium resembling foetal lung were not an infrequent sight.

5 - 18 weeks after operation

Lesions/

Lesions varied in intensity in the animals sacrificed from fifth week to the eighteenth week. Grossly, the wounds were marked by linear white scar on the surface of the lung. The low power photomicrographic appearance of these scars, both on the surface and in the interior of the lung, is illustrated in Figs. 162, 163, 165 and 166. The scarred areas contained collagen in which many haemosiderin-laden macrophages had been trapped, and cystic spaces with poorly defined lining were found. All the factors of regeneration were much more elaborate and advanced in these periods. Pleura was completely re-constituted. New bronchial buds and alveolar formation were prominent features. (Figs. 153-159, 164, 169, 171). Expansion of collapsed alveoli continued, if present, and the splitting of collagen to form new air spaces was seen in the subpleural regions under the new pleura. (Figs. 160, 161). The inflammatory reaction in the vicinity of the sutures was replaced by dense fibrous tissue (Fig. 164) which again had been gradually replaced by regenerated lung tissue. The fibrous zone around the suture material together with the suture was found to have been ultimately replaced by new lung tissue in 18 weeks. In one/

one case, however, the fibrous reaction was found predominent and the lung tissue was still imperfectly aerated around a suture (Fig. 167) though penetration of the area by epithelial buds from the periphery was in progress. (Figs. 168, 169).

Many arteries and veins were found, leading to the organised area or the scar of the wounds, in which the lumina had been largely obliterated by fibrous tissue giving rise to endarteritis obliterans. (Figs. 125, 153).

In some instances numerous bronchial buds lined by stratified squamous epithelium were found in the dense collagenous tissue of the scar. (Figs. 131, 132). This stratified atypical squamous metaplasia of the epithelium, often seen at the site of constricted parts of the parent bronchus, at first glance might suggest neoplasm. Instead, their presence seemed to be a protracted epithelial activity. There was no evidence of unrestricted growth to consider the lesion malignant and there was no indication of anaplasia or invasion.

It was in these various ways the regeneration of lung developed and proceeded until the whole wound was aerated, ultimately leaving behind/

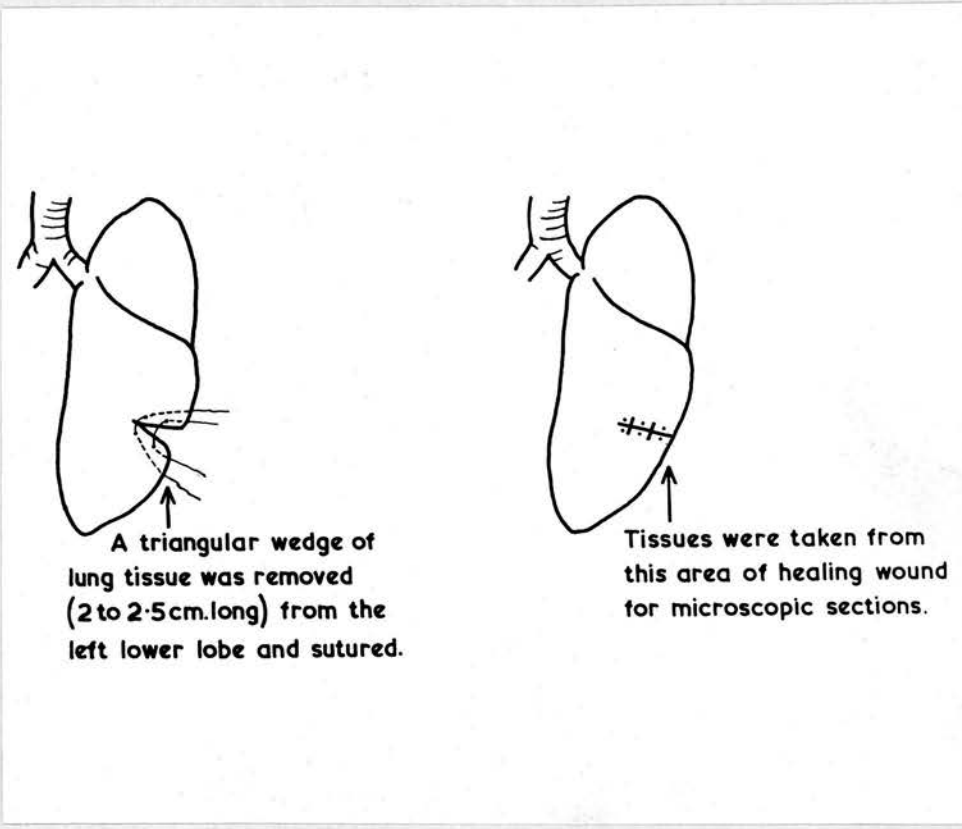

Fig.104 : Left lung (in cat). The area of excision is shown in the figure.

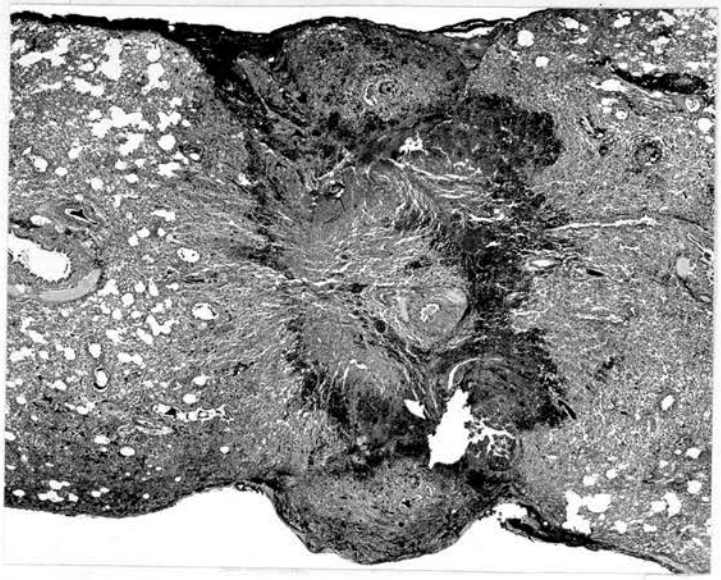

Fig.105 (Cat 1.) : Lung, 24 hours after operation. Blood-clot and local reaction in the wound. x 12.

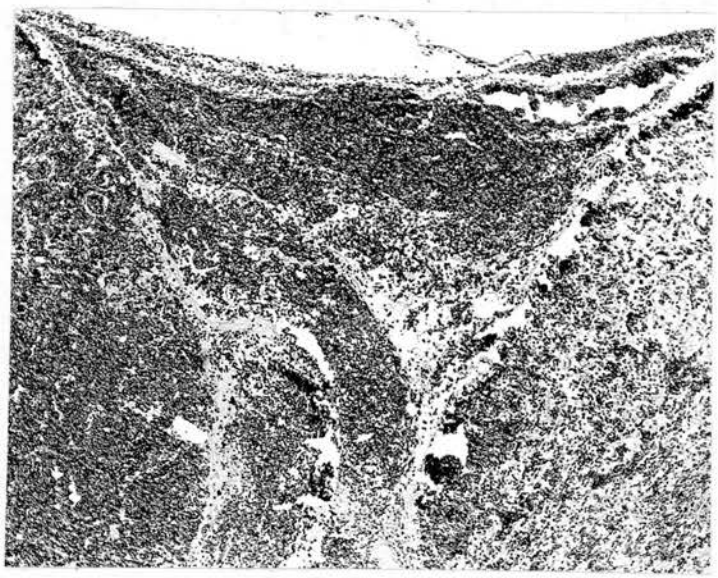

Fig.106 (Cat 1.) : High power of the above figure to show the gap of the wound filled up with blood. x 80.

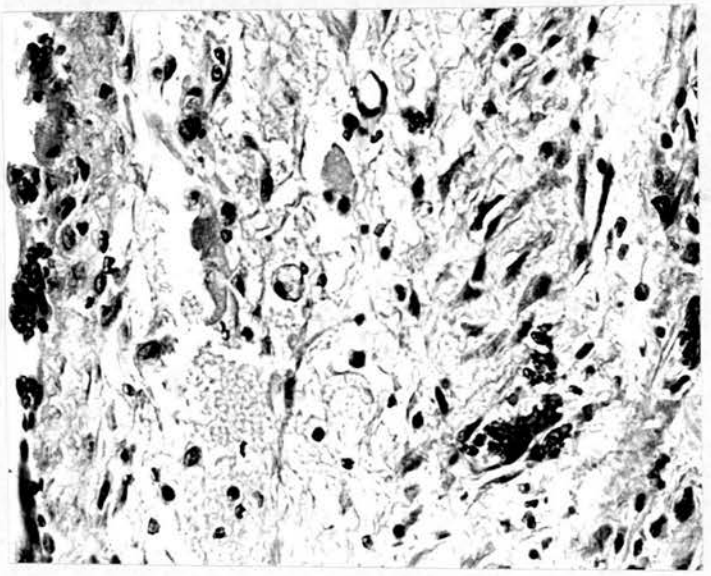

Fig.107 (Cat 2.) : Lung, 48 hours after operation. Early subpleural reaction beneath the existing pleura at the margin of the wound. x 400.

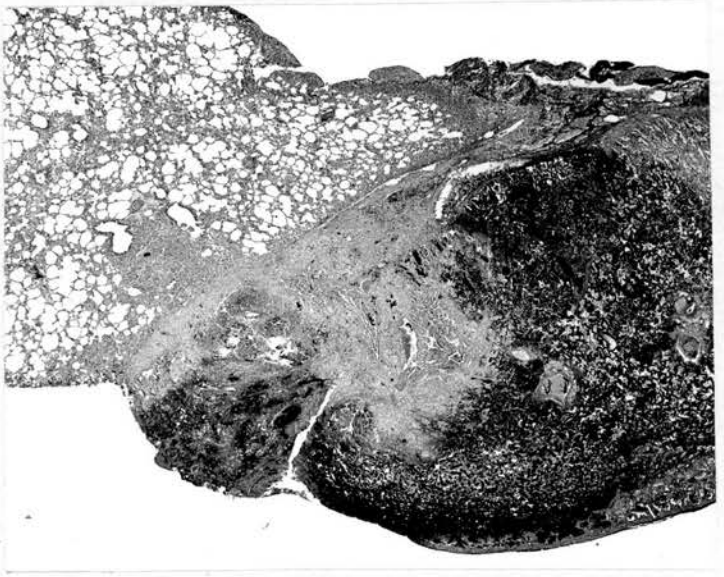

Fig.108 (Cat 3.) : Lung, 60 hours after operation. A central pale area of blood-clot with dark reactive lung tissue. x 11.

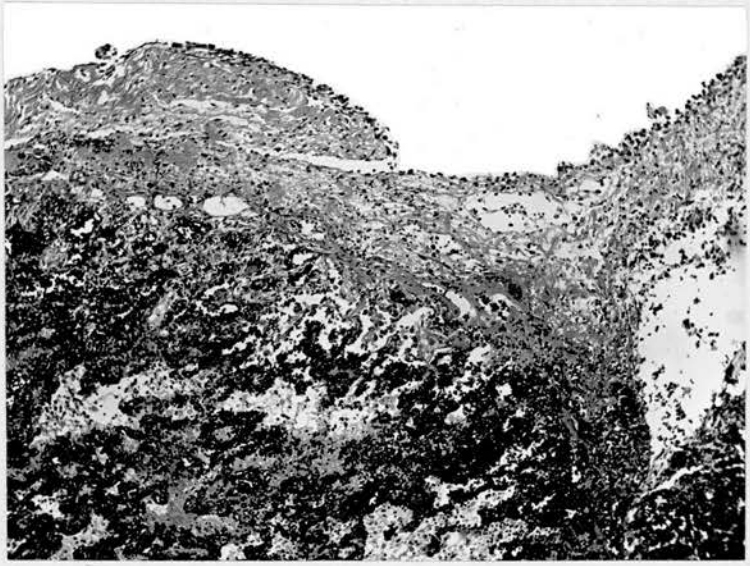

Fig.109 (Cat 3.) : At higher magnification. The pleural fibrinous reaction is well-shown with a dark line of newly-formed serosal cells. Many of the lung alveoli are dark from recent haemorrhage. Fibrin deposit(top) and inverted pleura into the wound(bottom right) are seen in the field. x 75.

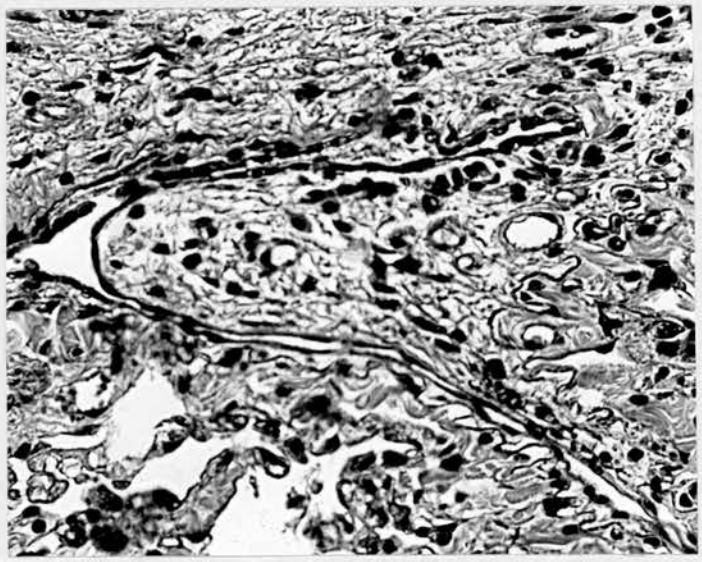

Fig.110 (Cat 3.) : Lung, 60 hours after operation. Serosal-cell reaction at the periphery of the fibrin deposited on the pleural surface. x 400.

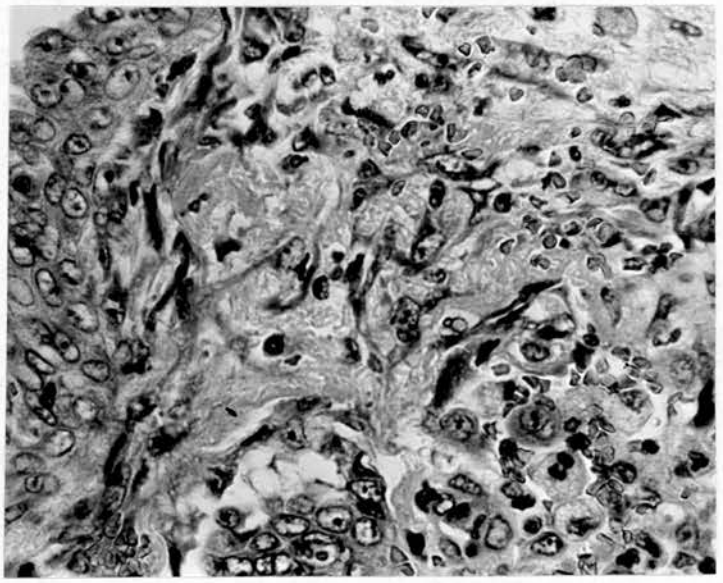

Fig.111 (Cat 3.) : Lung, 60 hours after operation. The figure shows a portion of bronchial mucosa(left) with proliferative change. In the remainder of the figure, fibroblasts and macrophages are at an early stage of activity. Several mitotic figures can be seen in the figure. x 550.

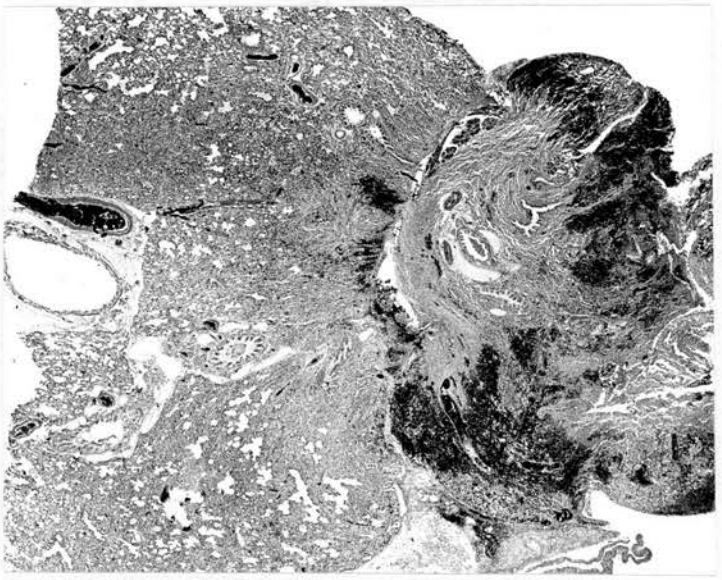

Fig.112 (Cat 4.) : Lung, 96 hours after operation, showing organisation of blood-clot and bronchial bud formation. x 12.

Fig.113 (Cat 4.) : Lung, 96 hours after operation. Vigorous serosal-cell reaction to fibrin occupying identation in superfices of wound. x 250.

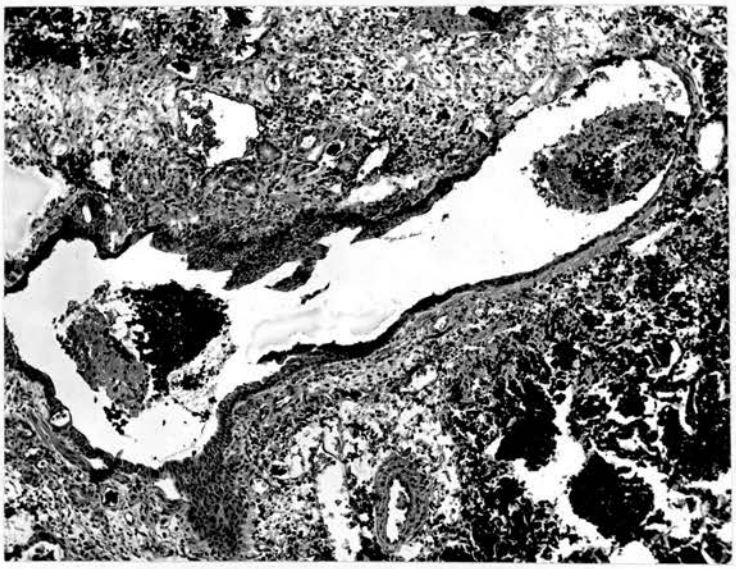

Fig.114 (Cat 4.) : Lung, 96 hours after operation, showing epithelial cell proliferation of a bronchus at the periphery of the wound. x 75.

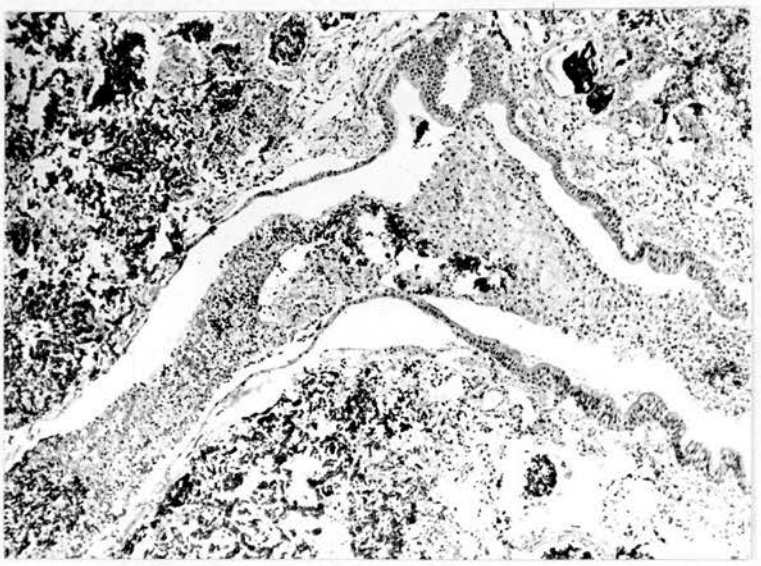

Fig.115 (Cat 4.) : Lung, 96 hours after operation, showing transformation of columnar epithelium of a bronchus into flattened cells at the margin of the wound. x 75.

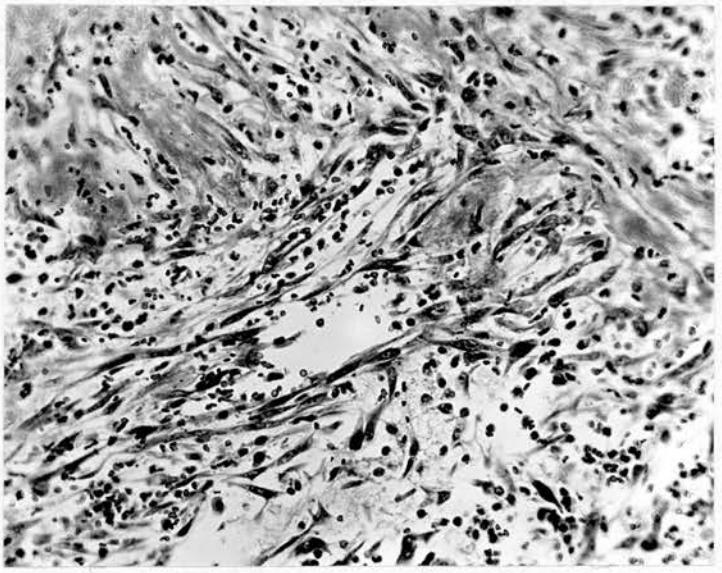

Fig.116 (Cat 4.) : Lung, 96 hours after operation, showing advanced fibroblastic reaction and capillary formation. x 250.

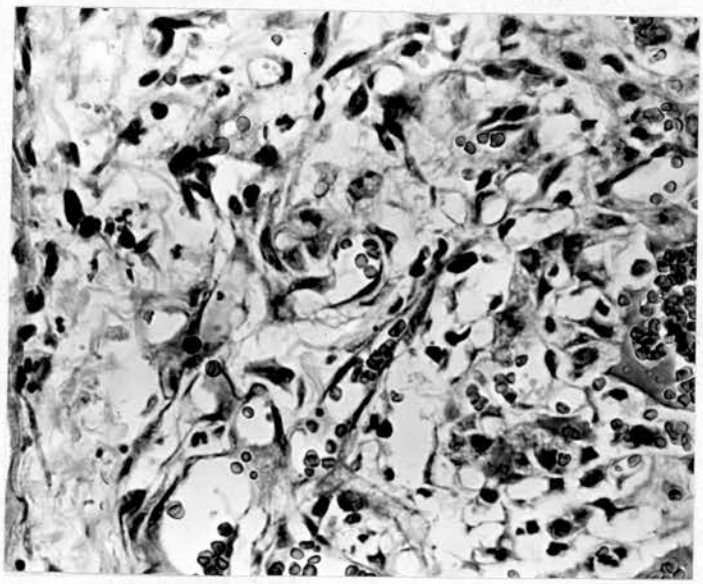

Fig.117 (Cat 4.) : Lung, 96 hours after operation. Subpleural fibroblastic reaction and capillary formation. Pleura on the left. x 500.

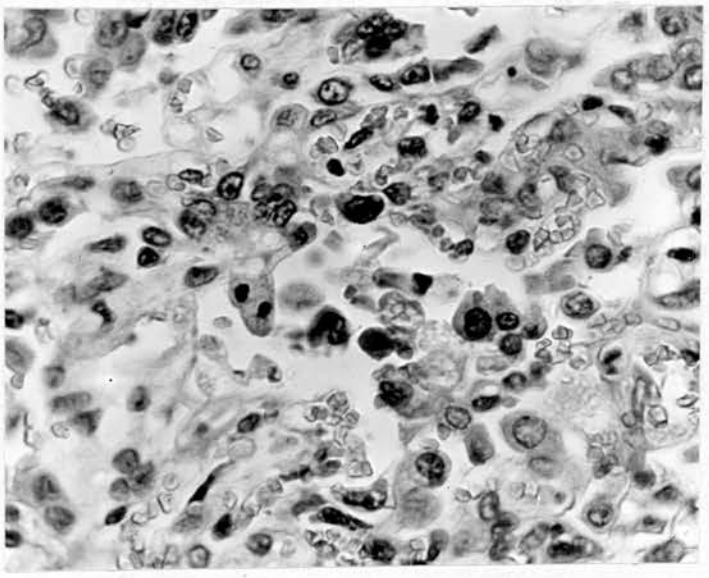

Fig.118 (Cat 4.) : Lung, 96 hours after operation, showing mitosis(centre) and phagocytosis in the organised area of the wound. x 550.

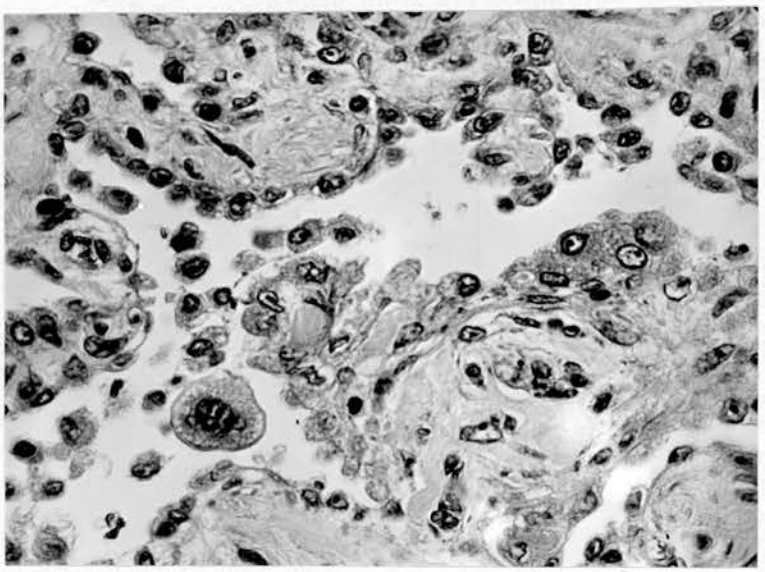

Fig.119 (Cat 4.) : Lung, 96 hours after operation. Phagocytosis in the regenerating area of the wound. Large macrophage cells are seen to bud out from alveolar wall. x 500.

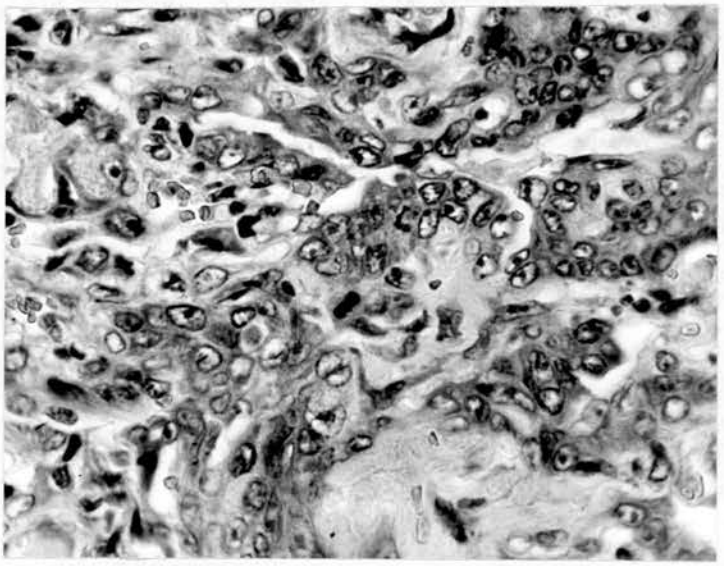

Fig.120 (Cat 4.) : Lung, 96 hours after operation, showing mitosis(centre) and phagocytosis. Substantial masses of epithelial cells are to be seen in the centre and to the right of the field. x 550.

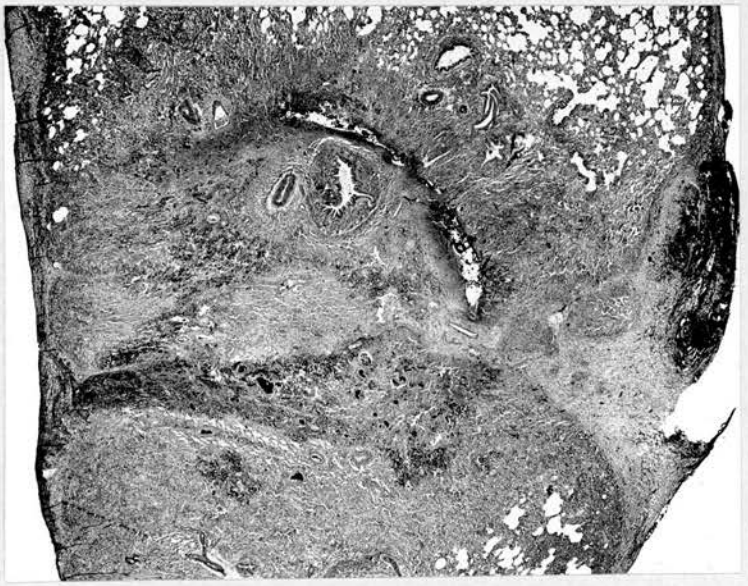

Fig.121 (Cat 6.) : Lung, 6 days after operation, showing bronchial budding and organisation of the whole area of blood-clot except a little on the right, under the pleura. x 12.

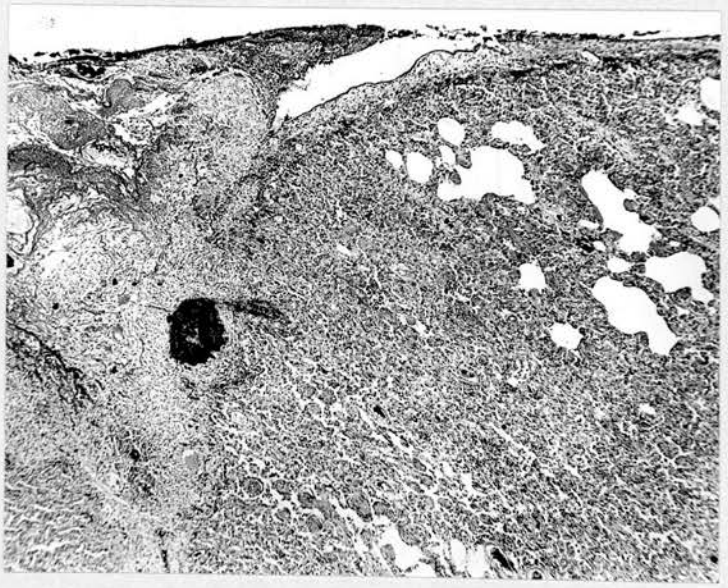

Fig.122 (Cat 6.) : Lung, 6 days after operation, showing the re-constitution of pleural membrane under the superficial mass of fibrin. x 35.

Fig.123 (Cat 6.) : Lung, 6 days after operation. Serosal-cell growing under the fibrin in spindle-shaped form. Collagen fibres are seen to separate pulmonary tissue (bottom left) from the overlying organised fibrin mass. x 500.

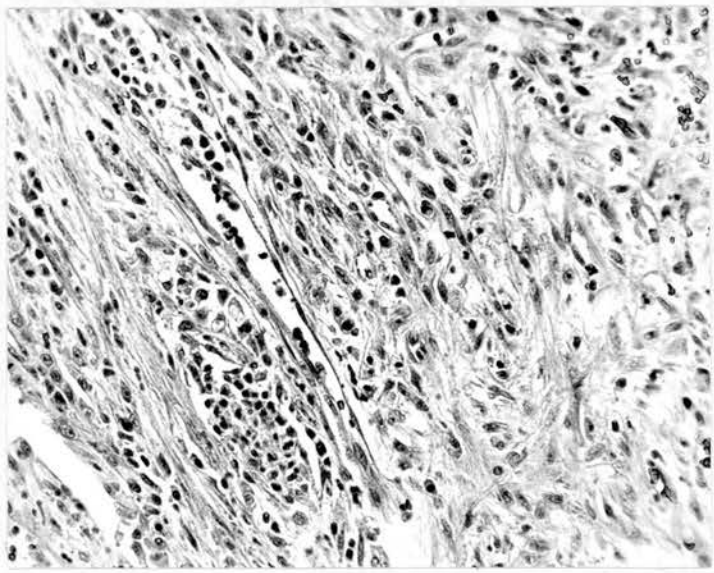

Fig.124 (Cat 6.) : Lung, 6 days after operation. New capillary formation and fibroblastic reaction in the organised part. x 275.

Fig.125 (Cat 6.) : Lung, 6 days after operation. Bronchial cell proliferation and epithelial bud formation(left) in an organised area of the wound. One of the arteries shows endarteritis obliterans. x 75.

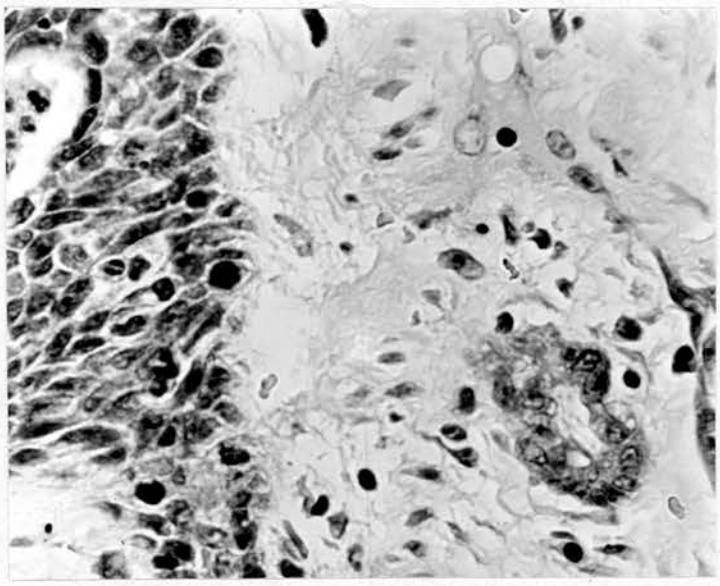

Fig.126 (Cat 6.) : High power of Fig.125, to show early mitotic activity in the proliferating bronchial epithelium and a canalised bronchial bud(right). x 550.

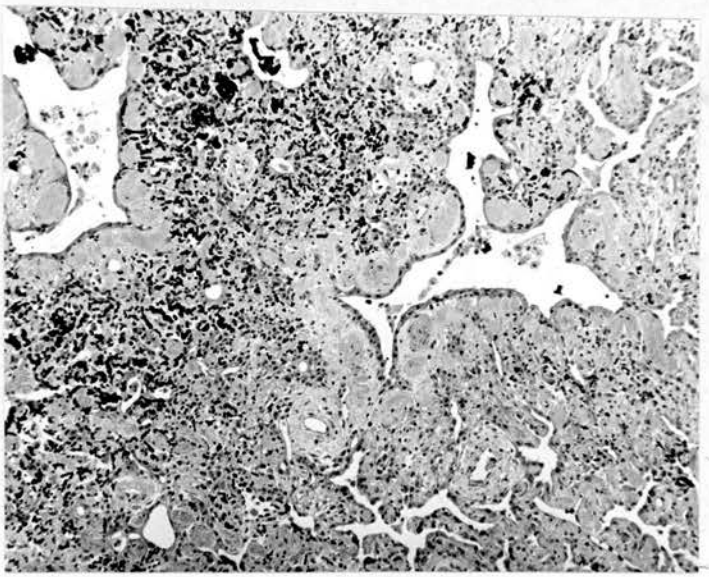

Fig.127 (Cat 6.) : Lung, 6 days after operation, showing the formation of new bronchi and alveoli, lined by cubical cells. x 75.

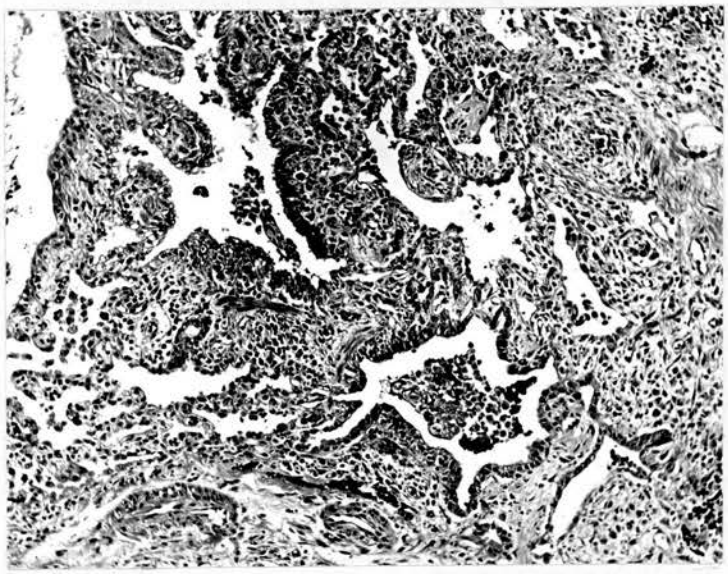

Fig.128 (Cat 6.) : Lung, 6 days after operation. Newly-formed bronchi with deeply-stained lining cells in a regenerating area of the wound. x 120.

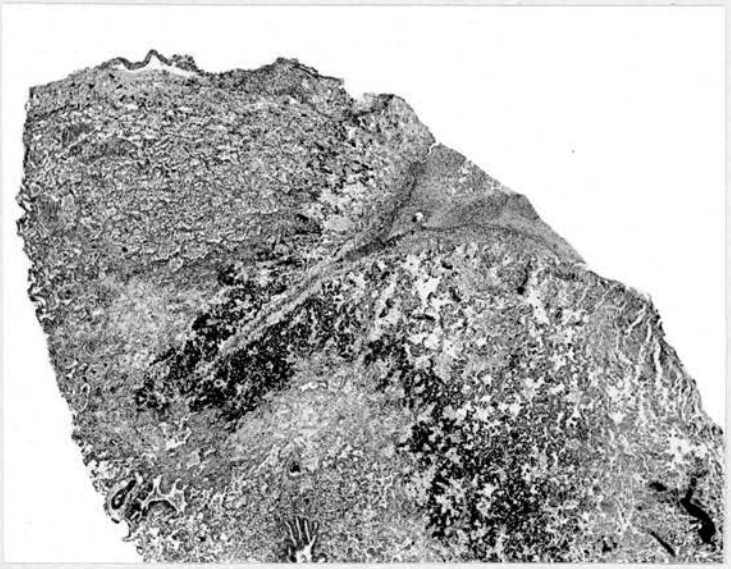

Fig.129 (Cat 7.) : Lung, 8 days after operation, showing pleura reconstituted under the debris of the wound. x 20.

Fig.130 (Cat 7.) : High power of Fig.129, to show the newly-formed pleural membrane and superficial debris of the wound. x 65.

Fig.131 (Cat 7.) : Lung, 8 days after operation. Bronchial bud formation and epithelial cell proliferation from a main bronchus at the margin of the wound. x 125.

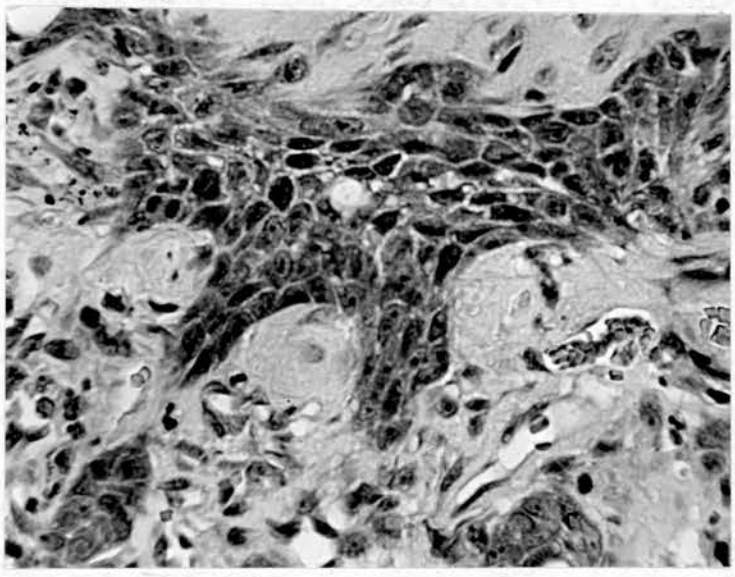

Fig.132 (Cat 7.) : High power of Fig.131, to show mitotic activity(left) in bronchial buds. The cells have assumed a stratified squamous appearance. x 525.

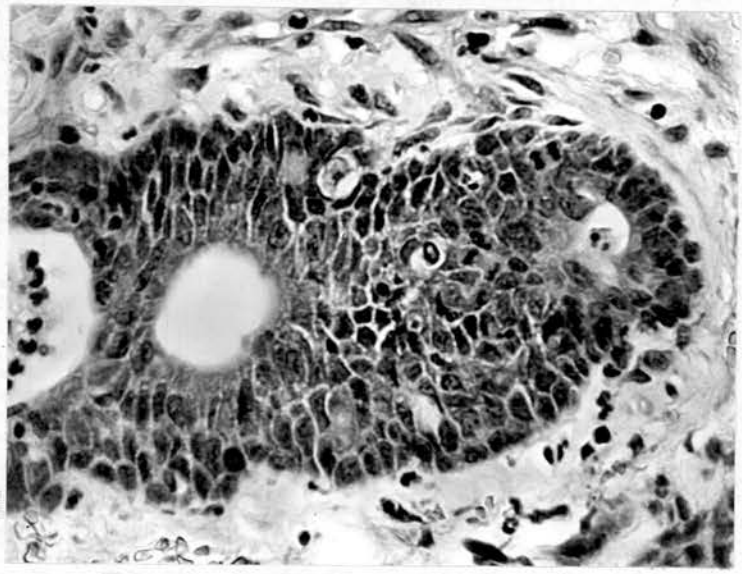

Fig.133 (Cat 7.) : High power of Fig.131, to show mitosis and canalisation of bronchial buds. The stratified squamous appearance is well shown. x 525.

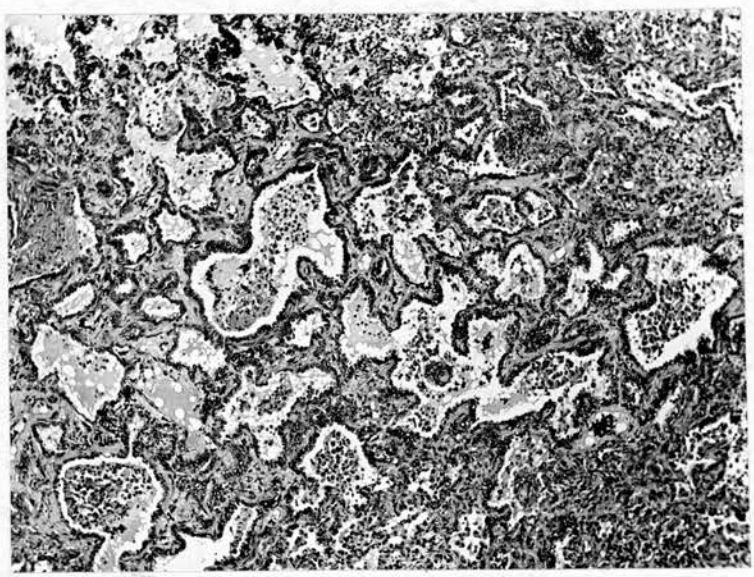

Fig.134 (Cat 7.) : Lung, 8 days after operation. An area of lung tissue to show the new alveoli which have formed through bronchial budding. x 75.

Fig.135 (Cat 8.) : Lung, 11 days after operation, showing regeneration of pleura and re-aeration of lung tissue. x 15.

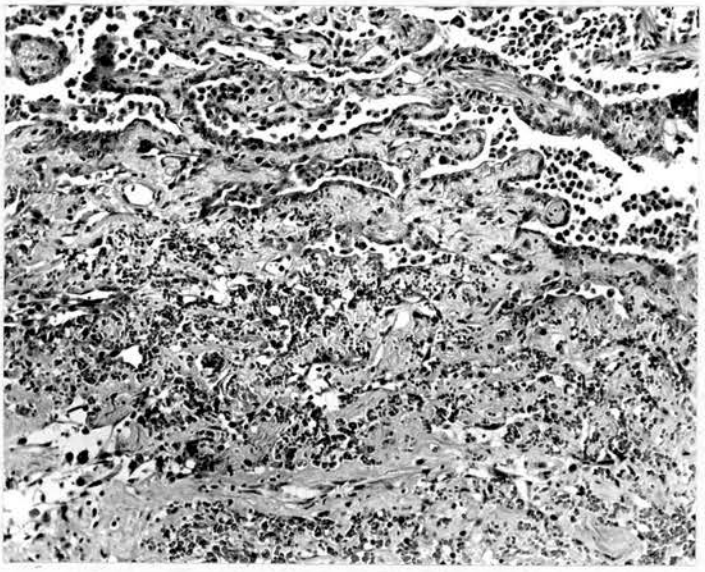

Fig.136 (Cat 8.) : Lung, 11 days after operation. The newly-formed bronchi have grown into alveoli with macrophages in their lumen. x 125.

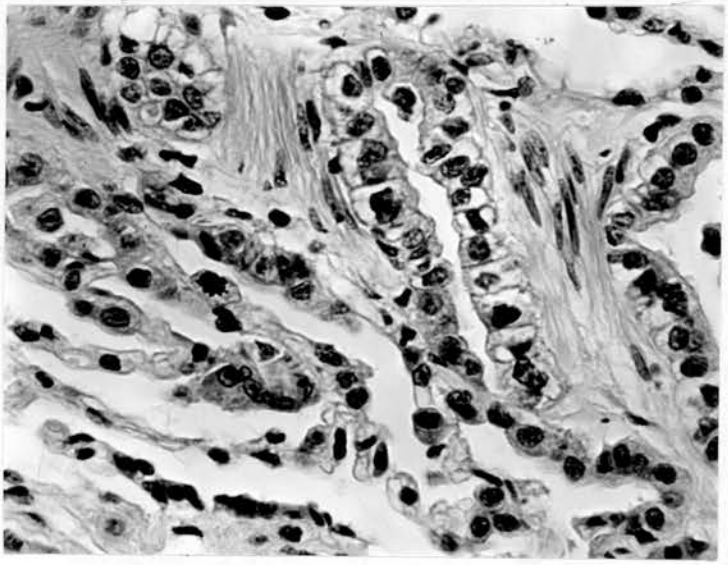

Fig.137 (Cat 8.) : High power of Fig.136, to show regenerating alveoli lined by cubical cells with mitoses(left centre). x 525.

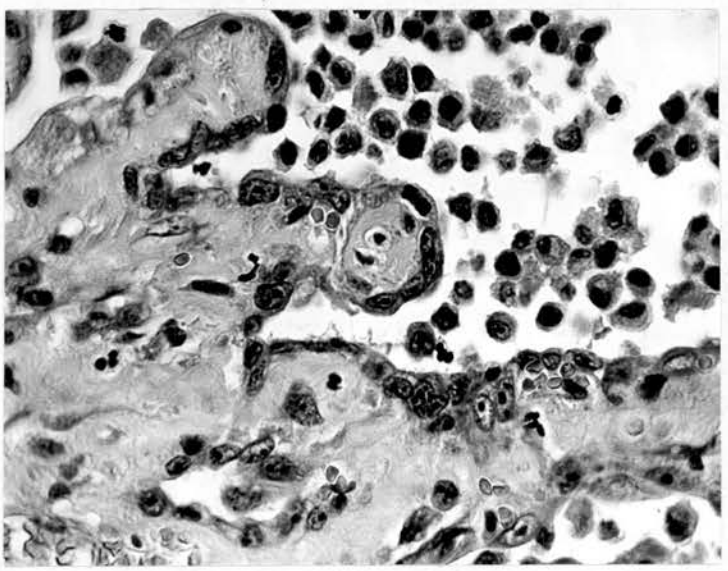

Fig.138 (Cat 8.) : High power of Fig.136, to show mitotic activity in the lining cells of new alveoli. x 525.

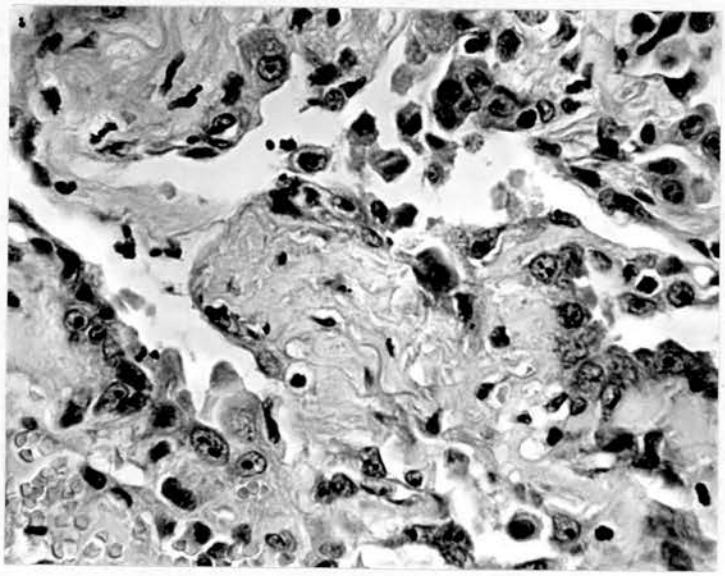

Fig.139 (Cat 8.) : Lung, 11 days after operation. Newly-formed alveoli and mitoses are seen in the regenerating lung tissue. x 525.

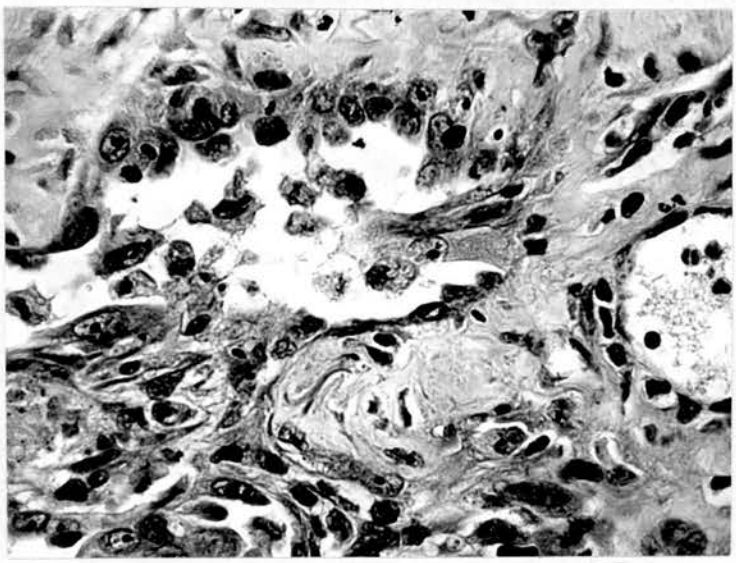

Fig.140 (Cat 8.) : Mitoses are seen in the lining cells of the regenerating alveoli of the same case as above. Large round macrophage cells are seen to arise from the alveolar lining. x 525.

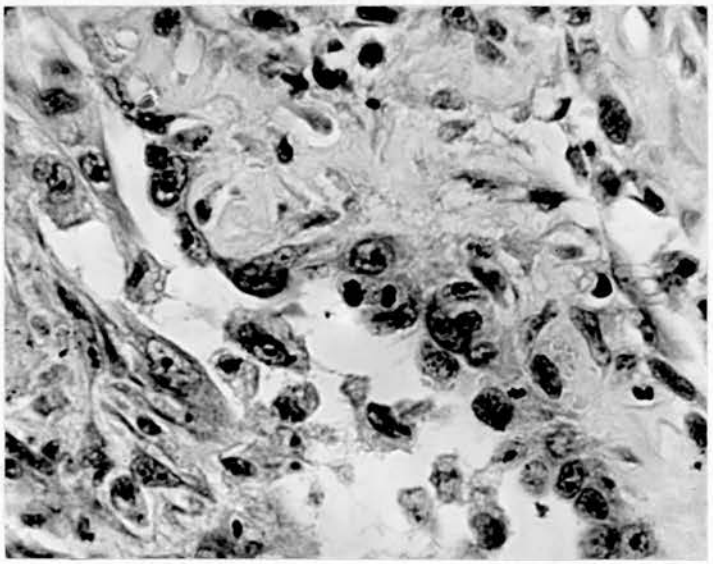

Fig.141 (Cat 8.) : Lung, 11 days after operation. Mitotic activity is seen in the lining cell of a new alveoli. x 650.

Fig.142 (Cat 11.) : Lung, 12 days after operation. Re-formed pleural membrane on the surface portion of the wound. x 70.

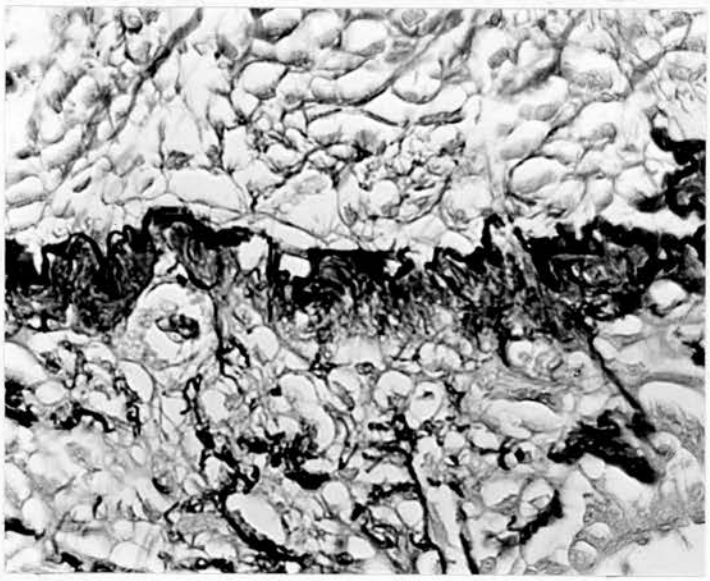

Fig.143 (Cat 11.) : Lung, 12 days after operation. New fibro-elastic pleural membrane connected with the elastic tissue of marginal alveoli. x 500.

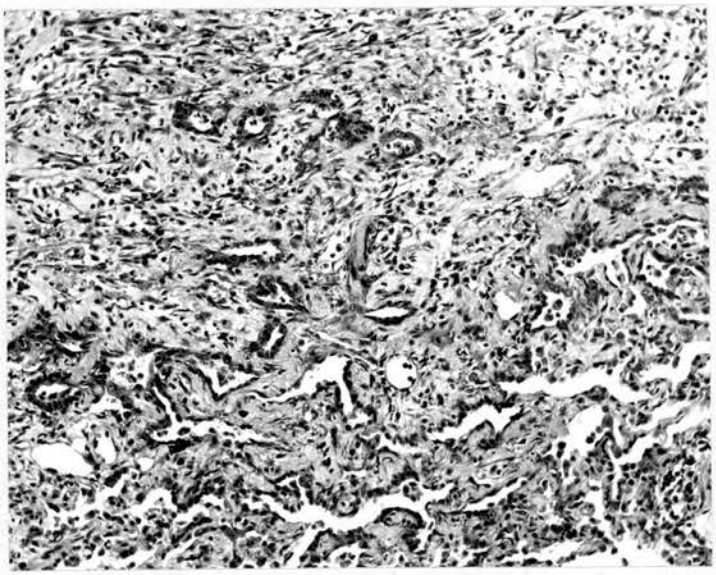

Fig.144 (Cat 11.) : Lung, 12 days after operation, showing bronchial buds and alveolar formation at the margin of the wound. x 125.

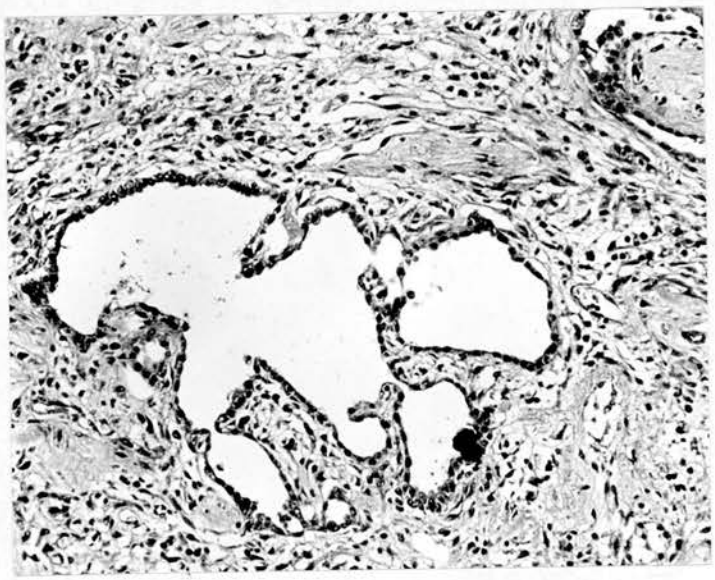

Fig.145 (Cat 11.) : Lung, 12 days after operation. Re-formed alveoli lined by cubical cells in the fibrosed part of the wound. x 170.

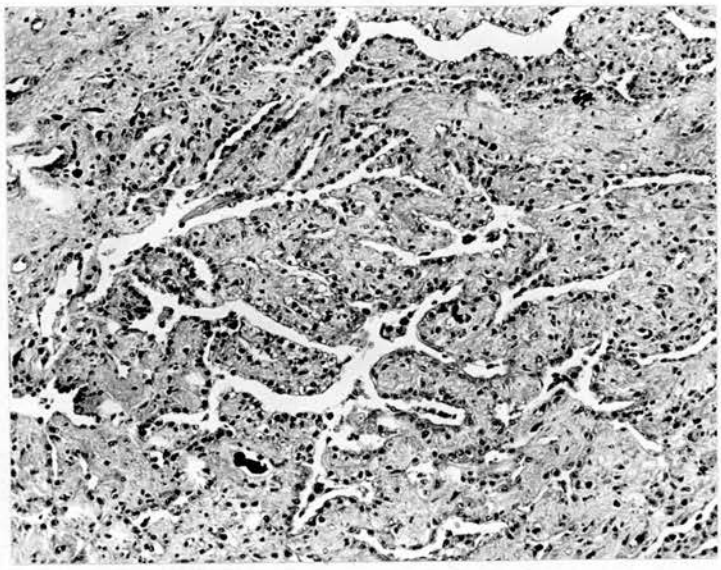

Fig.146 (Cat 11.) : Lung, 12 days after operation, showing regenerated bronchi and alveoli in the fibrous tissue. x 140.

Fig.147 (Cat 12.) : Lung, 20 days after operation, showing regeneration of lung tissue. Bronchial buds and newly-formed alveoli are seen in the dense area. x 12.

Fig.148 (Cat 12.) : Lung, 20 days after operation. Regenerating bronchi and alveoli are seen in the fibrosed area. x 125.

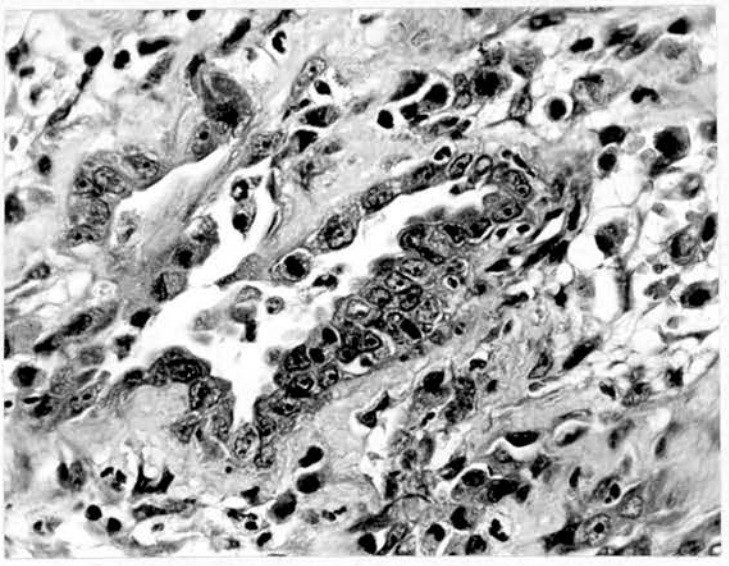

Fig.149 (Cat 12.) : High power of Fig.147, to show a newly-formed bronchus which is partly lined by columnar cells. Mitoses are seen in the lining epithelium. x 525.

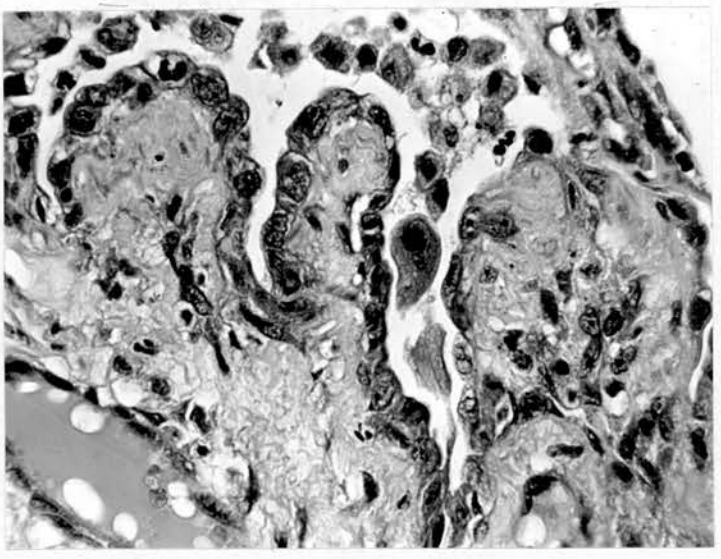

Fig.150 (Cat 12.) : High power to show cubical cell lining of new alveoli with mitoses. x 525.

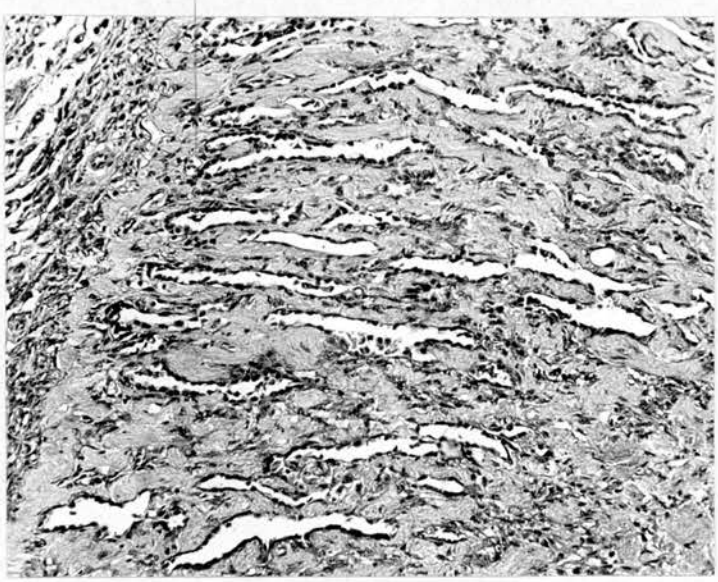

Fig.151 (Cat 12.) : Lung, 20 days after operation. Re-expanded alveoli bordering immediately traumatised area or blood-clot of wound. Pleura on left. x 120.

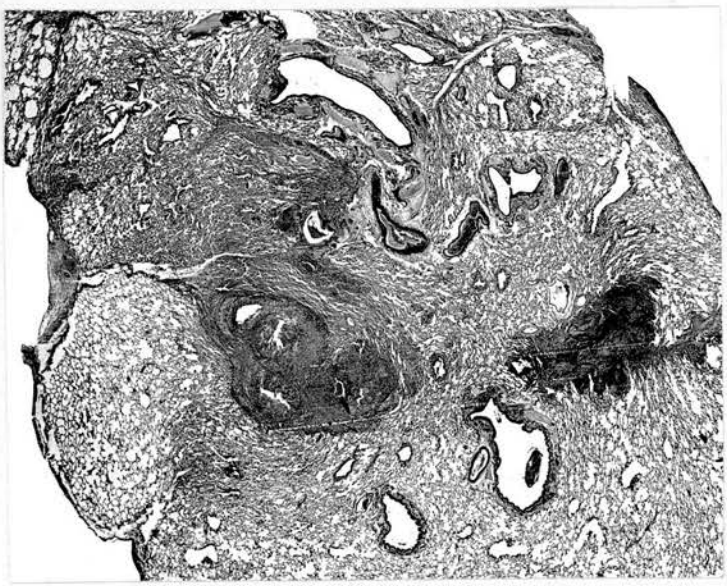

Fig.152 (Cat 13.) : Lung, 35 days after operation, showing almost complete repair of the wound by regeneration of lung tissue with re-formation pleural surface. x 7.

Fig.153 (Cat 13.) : Lung, 35 days after operation, showing bronchial budding and formation of new bronchi from a viable bronchus(bottom left) at the margin of the wound. Two arteries show endarteritis obliterans. x 150.

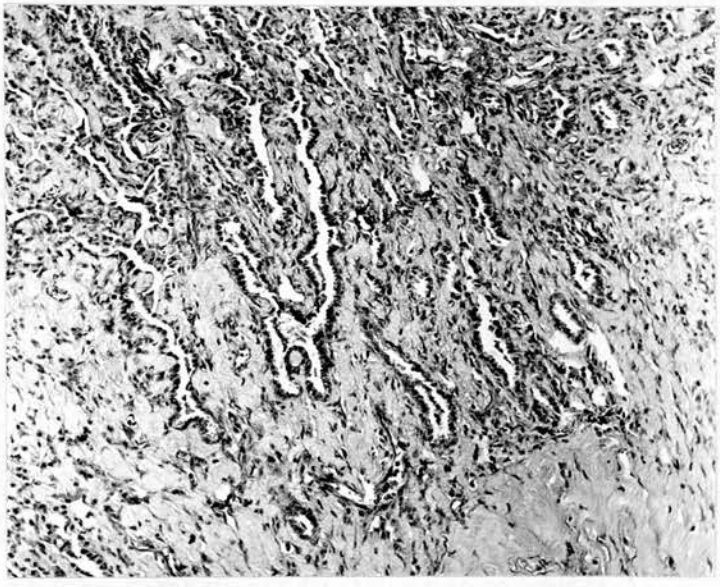

Fig.154 (Cat 13.) : Lung, 35 days after operation. The newly-formed bronchi are seen penetrating the fibrous tissue of the wound. x 125.

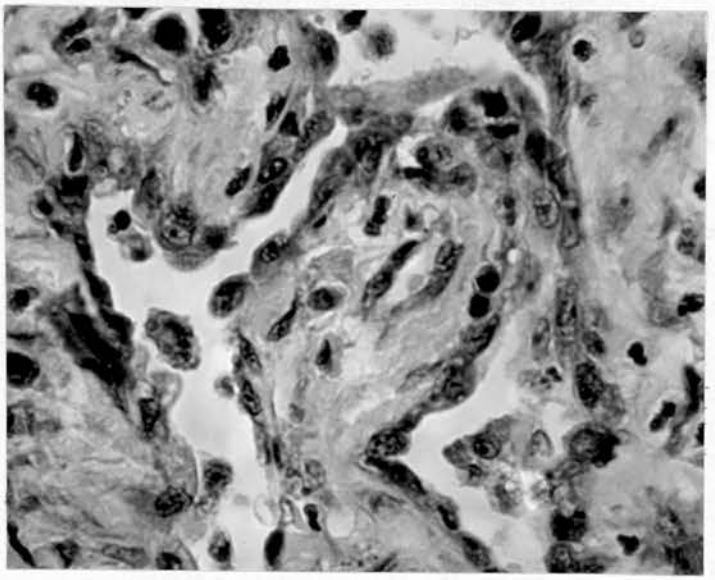

Fig.155 (Cat 13.) : High power view of regenerating alveoli from Fig.152, showing the lining cells of new alveoli - some are cubical and some flattened. x 650.

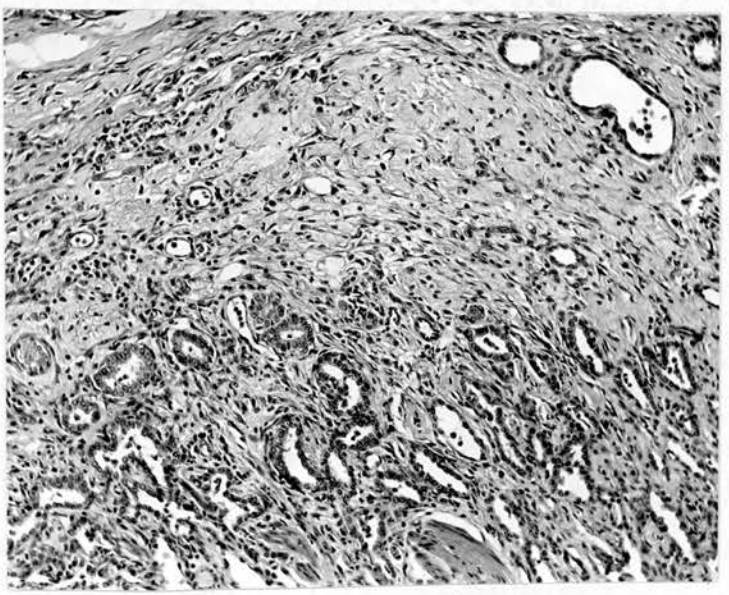

Fig.156 (Cat 13.) : Lung, 35 days after operation. New bronchi and alveoli at the margin of the wound. x 125.

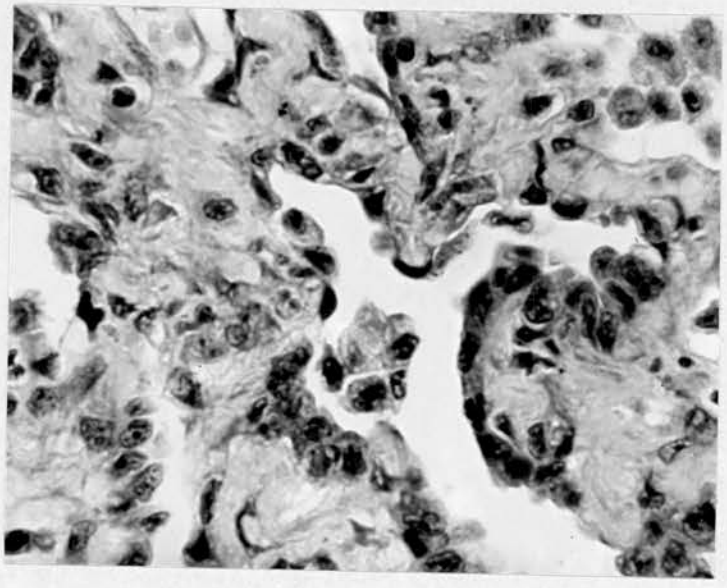

Fig.157 (Cat 13.) : High power view of a newly-formed bronchus and alveoli to show cellular lining. x 650.

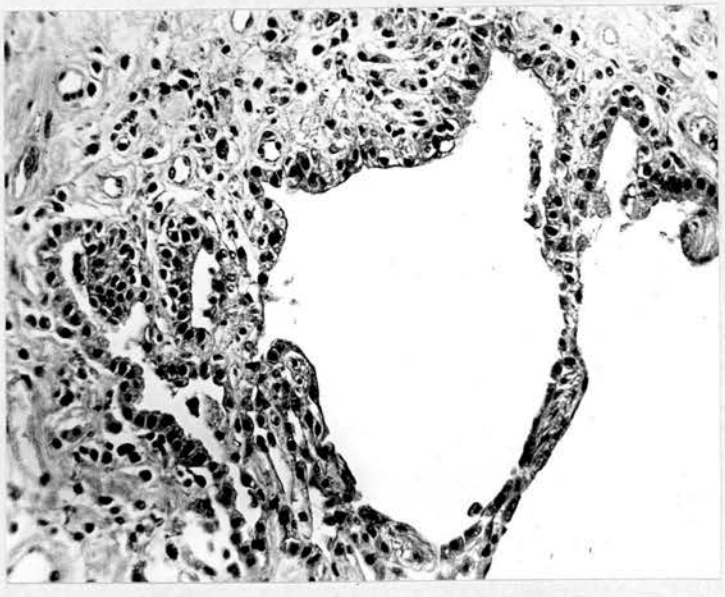

Fig.158 (Cat 13.) : Lung, 35 days after operation, showing the lining cells of a new alveoli. Two alveoli (left) are lined by cubical cells. x 300.

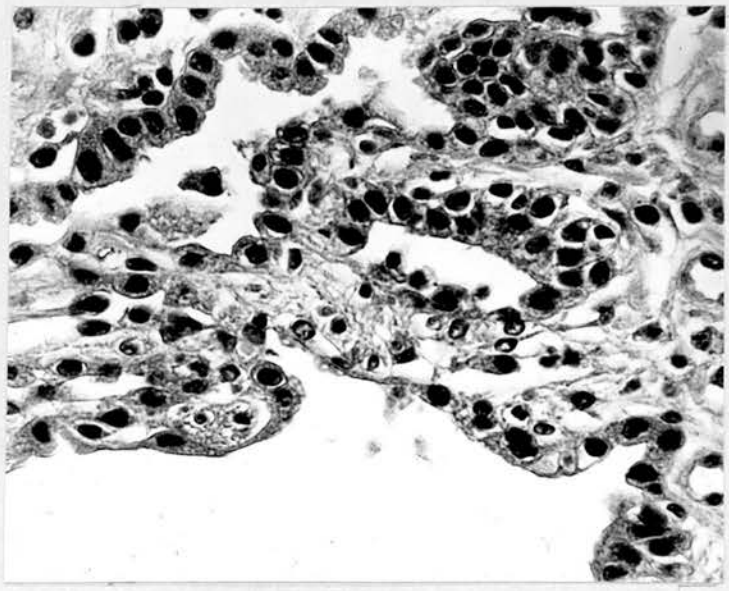

Fig.159 (Cat 13.) : High power of Fig.158, to show the conversion of cubical cells into flattened epithelium, typical of normal alveoli. x 600.

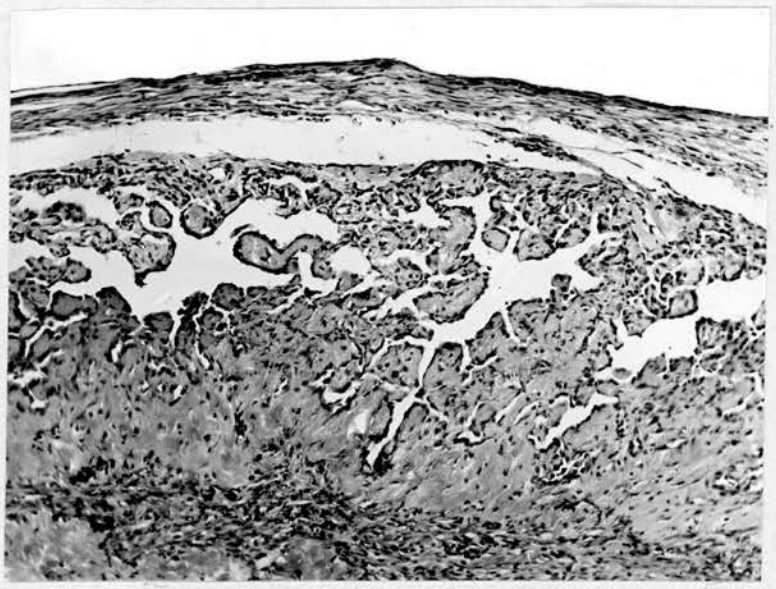

Fig.160 (Cat 13.) : Lung, 35 days after operation. Re-aeration of lung tissue by regeneration in the sub-pleural region. The surface fibrin is organised and separated from the new pleural membrane. x 110.

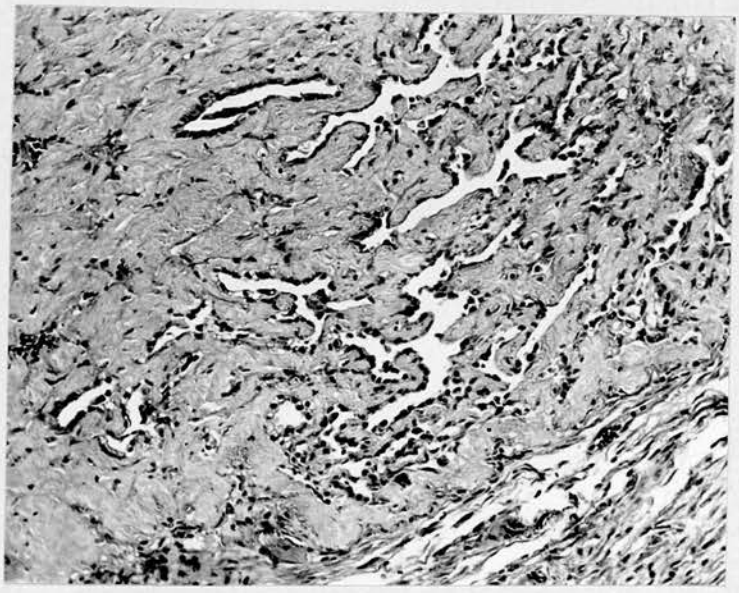

Fig.161 (Cat 13.) : Lung, 35 days after operation. New subpleural air spaces, lined by cubical and flattened cells. x 150.

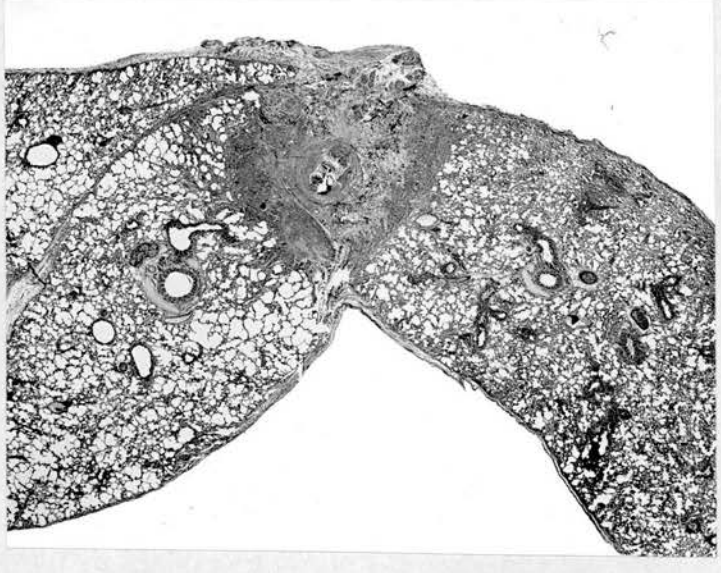

Fig.162 (Cat 14.) : Lung, 90 days after operation, showing repair of the wound by regeneration. x 7.

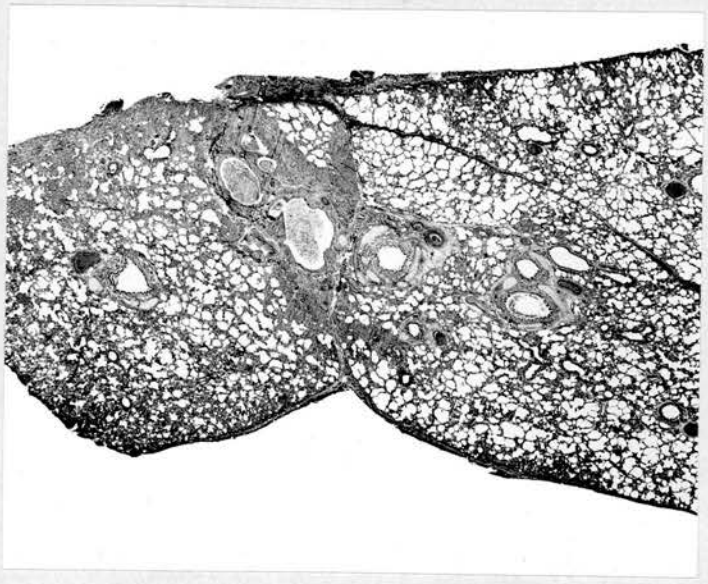

Fig.163 (Cat 15.) : Lung, 90 days after operation, The wound almost completely aerated by regeneration of lung tissue. x 7.

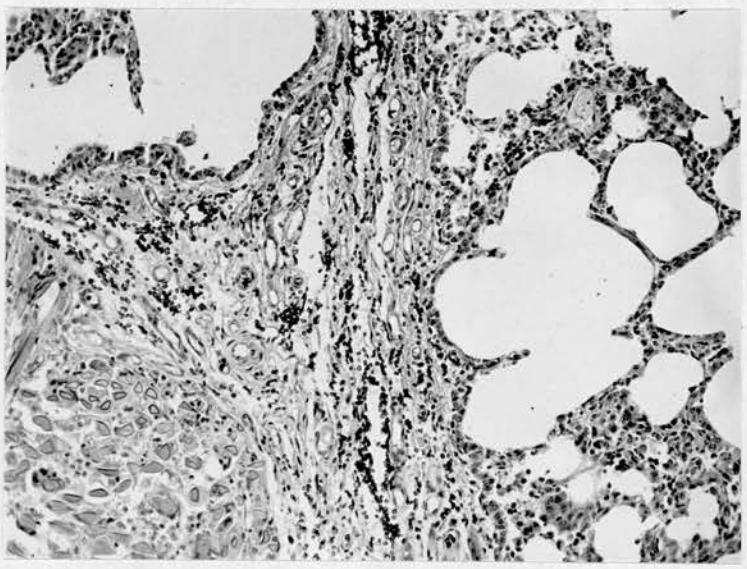

Fig.164 (Cat 14.) : Lung, 90 days after operation. Newly-formed alveoli(top left). The suture material still remains unabsorbed and is surrounded by fibrous tissue. x 135.

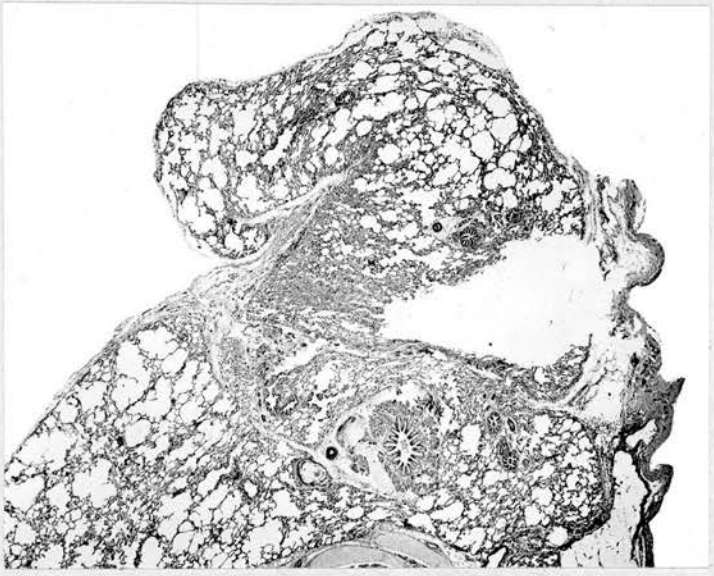

Fig.165 (Cat 16.) : Lung, 130 days after operation, showing almost complete repair by regeneration of lung tissue. x 13.

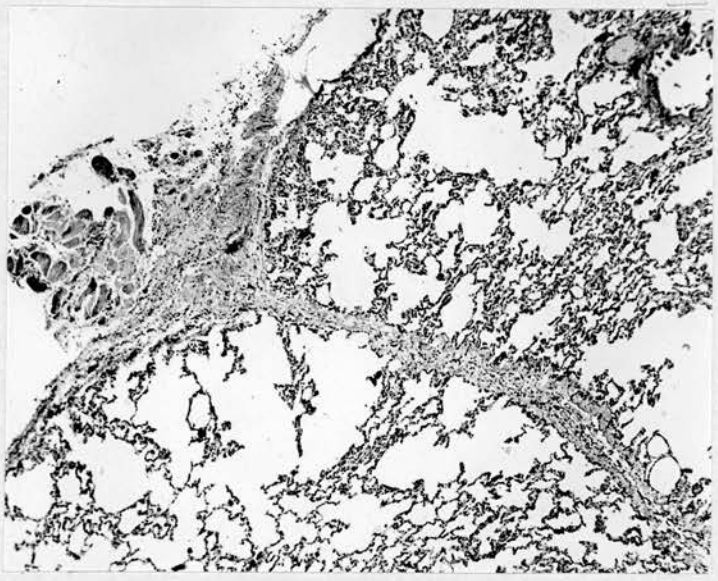

Fig.166 (Cat 17.) : Lung, 130 days after operation, showing complete repair by regeneration of lung tissue. Only a thin linear scar is left. x 45.

Fig.167 (Cat 18.) : Lung, 130 days after operation, showing much less advanced in repair. Partial regeneration has occurred around the suture material which is surrounded by fibrous tissue. x 13.

Fig.168 (Cat 18.) : High power of Fig.167, to show new bronchi and alveoli invading the fibrous tissue around the suture material. x 130.

Fig.169 (Cat 18.) : High power of the dense fibrous area from the margin of Fig.167, to show the invading epithelial projection from the margin. x 135.

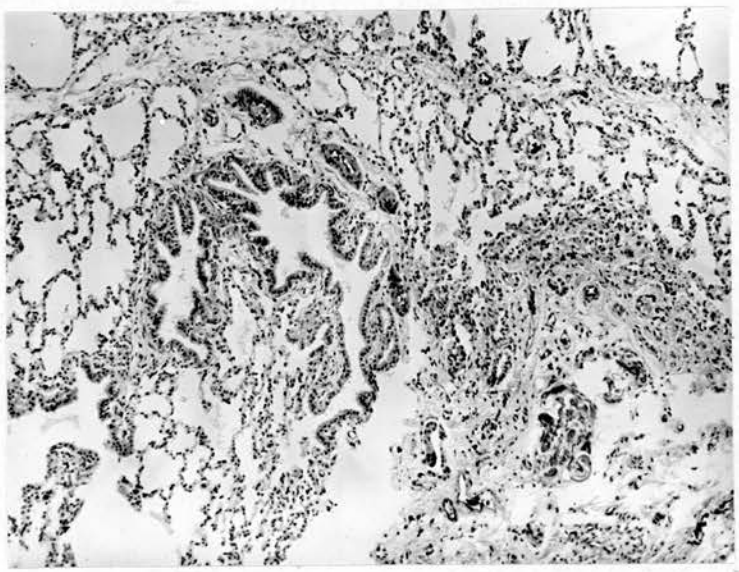

Fig.170 (Cat 16.) : Lung, 130 days after operation, showing how the suturing material with the surrounding fibrous tissue is being almost completely absorbed and replaced by regenerated lung tissue. x 85.

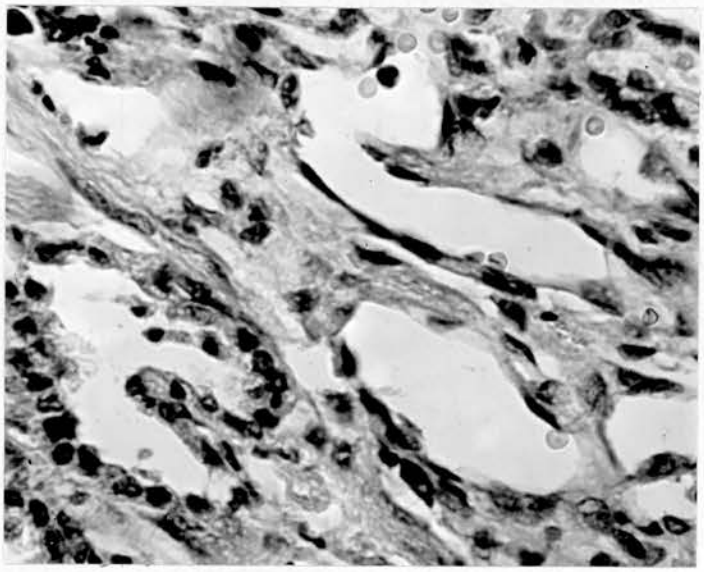

Fig.171 (Cat 17.) : Lung, 130 days after operation. New alveoli lined by cubical and flattened cells. x 650.

hind a thin band of collagenous elastic tissue as a remnant of the scar of the wound. (Fig. 166).

HISTOCHEMICAL REACTIONS OF THE REGENERATING PULMONARY TISSUE

Normal lung: Microscopical examination of sections of normal lung of catshowed positive enzyme reactions for non-specific esterase and alkaline phosphatase in bronchial epithelium which was continued into the smaller divisions of bronchi though less intensely. (Figs. 172, 173). The few lining cells of normal alveoli were virtually negative for alkaline phosphatase and esterase as indicated by one or two brick-red and pinkish-red dots in the scanty lining cells. Acid phosphatase reaction was absent in the normal alveolar walls.

With regard to acid phosphatase, the bronchial epithelium was somewhat irregular in its reaction, the epithelium of some of the smaller bronchi being positive and others negative, (Fig. 174), though bronchial cartilage was always positive.

As for R.N.A. and D.N.A., the epithelium of the large bronchi was normally strongly positive/

tive (Fig. 175), the smaller bronchi less so.

Healing Lung : non-specific esterase and alkaline phosphatase were both definitely positive (as compared with normal controls) in the bronchial epithelium in the periphery of wounds of lung about 96 hours after the injury (Figs. 176, 177-8 days); earlier than this, no change was found. Positive enzyme staining reactions were given also by the large macrophage cells in the lung alveoli and also by swollen alveolar lining cells. (Figs. 178 - 183). These reactions were strikingly positive not only in the bronchial epithelium adjacent to the wound but also in the bronchial buds, whether canalised or closed. (Figs. 184 - 189).

R.N.A. and D.N.A. reactions remained positive in these regenerating cells of bronchial buds and new alveoli whenever examined in the course of the healing process over many weeks. (Figs. 190 - 192). Some scattered cells, in the organised areas of the wound gave positive R.N.A. and D.N.A. staining reactions throughout thehealing phase indicating the presence of few pus cells in those areas. (Figs. 193, 194).

The acid phosphatase behaved similarly (Figs. 195, 196), though it was noticed that it returned/

returned more readily to the normal intensity once the bronchial buds were canalised and established.

In all cases the cubical cells of the newly-formed bronchial buds and alveoli were always more strongly positive than the flattened elongated cells. (Figs. 197-200, 190).

Alcian blue reaction for mucopolysaccharide was constantly positive in the mucous cells of bronchial epithelium when actively secreting. Bronchi trapped in the scar tissue, however, did not give a positive alcian blue reaction. Fibroblastic areas gave an alcian blue reaction which indicated the presence of acid-mucopolysaccharide in fibroblast cells and served as control. (Fig. 201). Epithelium of the regenerated bronchial buds and the lining cells of alveoli did not stain with alcian blue.

SUMMARY (Lung wound)

Repair of experimental wounds of mammalian lung has been studied. From such studies the following facts have been observed :

(1) The production of wound of lung tissue in adult cats is followed by subsequent healing with the formation of pneumonic tissues/

tissues in the organised part of the wound.

(2) The reactive processes assume two main forms which lead to the restoration of the lost part of the lung by parenchymal regeneration: firstly, the surviving bronchial epithelium near the wound margin begins to proliferate and undergoes rapid multiplication to form bronchial buds, and, secondly, these bronchial buds, under the influence of a kind of directional impulse from the wound itself, invade the organised area of the lesion and re-aerate the part, a process which may persist for 3 - 4 months after the original injury.

(3) The re-expansion of collapsed alveoli and the formation of new air spaces by splitting of collagen of the scar of the wound also contribute to the above major regenerative process.

(4) The re-constitution of the pleural membrane over the traumatised area takes place, simultaneously with the process of regeneration of the lung and is complete in 12 - 15 days.

Factors/

Factors governing the above processes have been discussed in the chapter of general discussion of this present work.

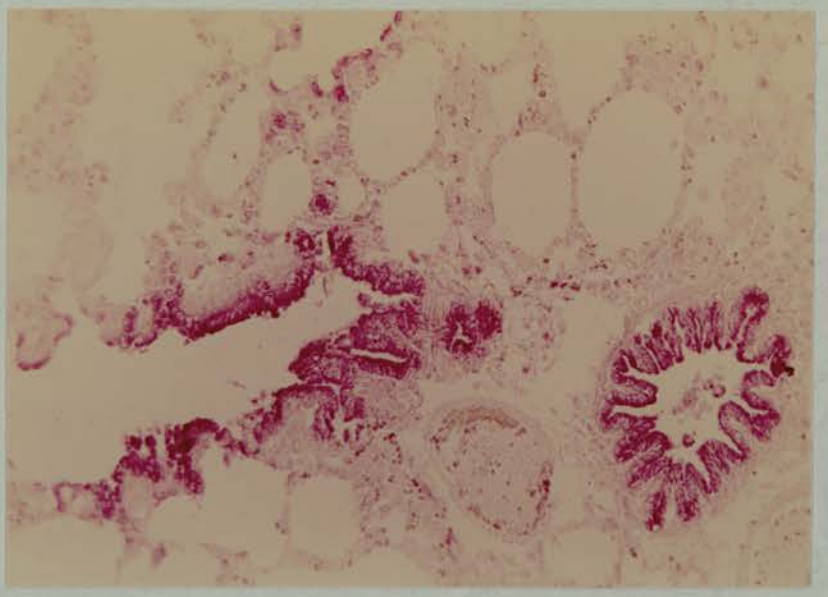

Fig.172 (Cat 14.) : Normal lung. Non-specific esterase reaction, positive in the bronchial epithelium. Smaller divisions of the bronchus show less intensity. x 150.

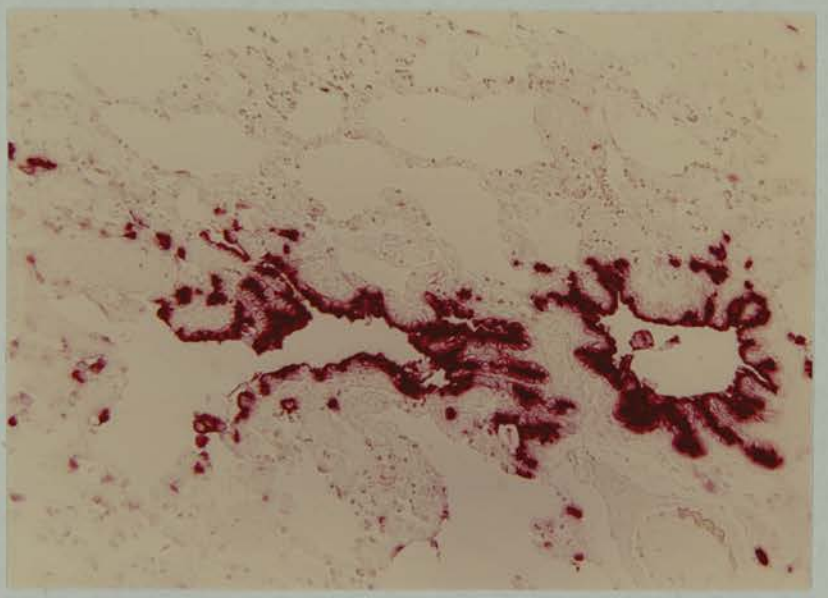

Fig.173 (Cat 14.) : Normal lung. Alkaline phosphatase reaction in the bronchial epithelium. The smaller divisions show less intense reaction. x 150.

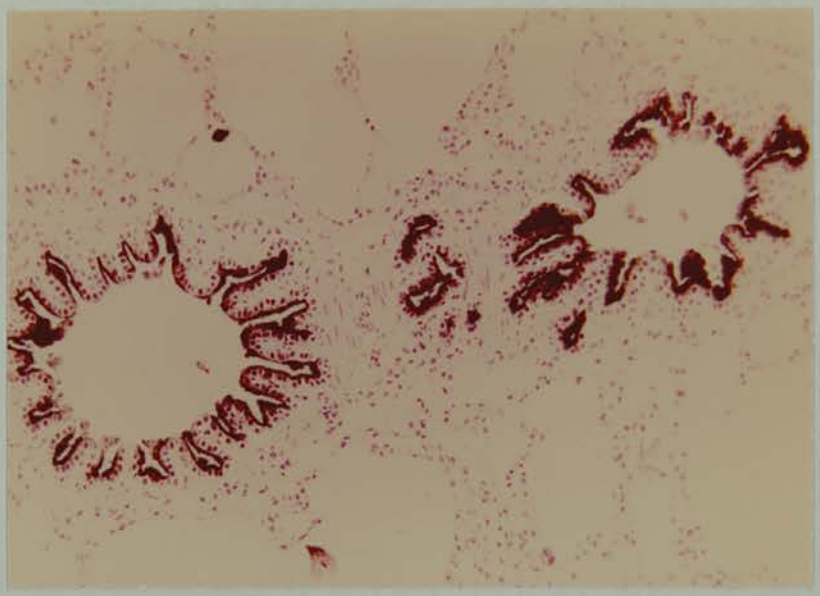

Fig.174 (Cat 14.) : Normal lung. Acid phosphatase reaction. Bronchial epithelium somewhat irregular in its reaction, some portions being positive and others negative. x 150.

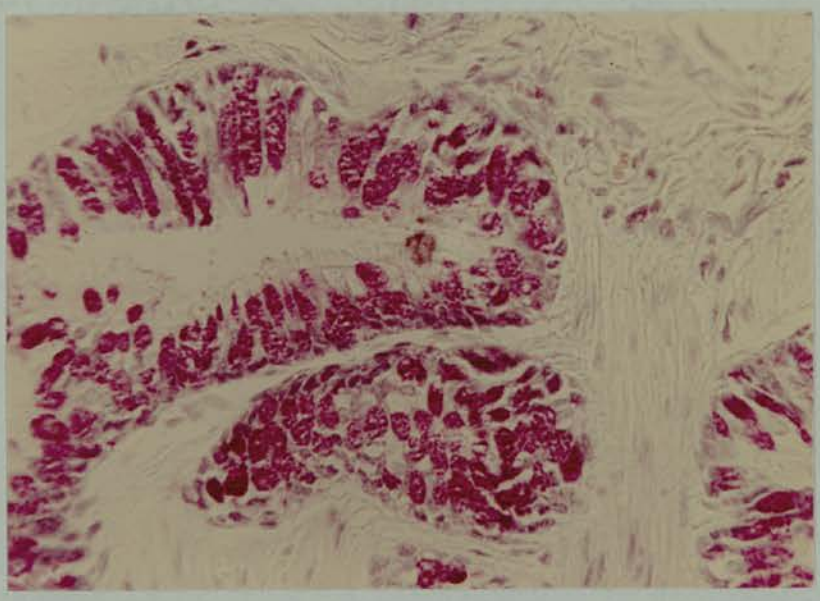

Fig.175 (Cat 14.) : Normal lung. The bronchial epithelium giving positive staining reactions for R.N.A. & D.N.A. Pinkish cytoplasma - R.N.A., and green nuclei - D.N.A. x 450.

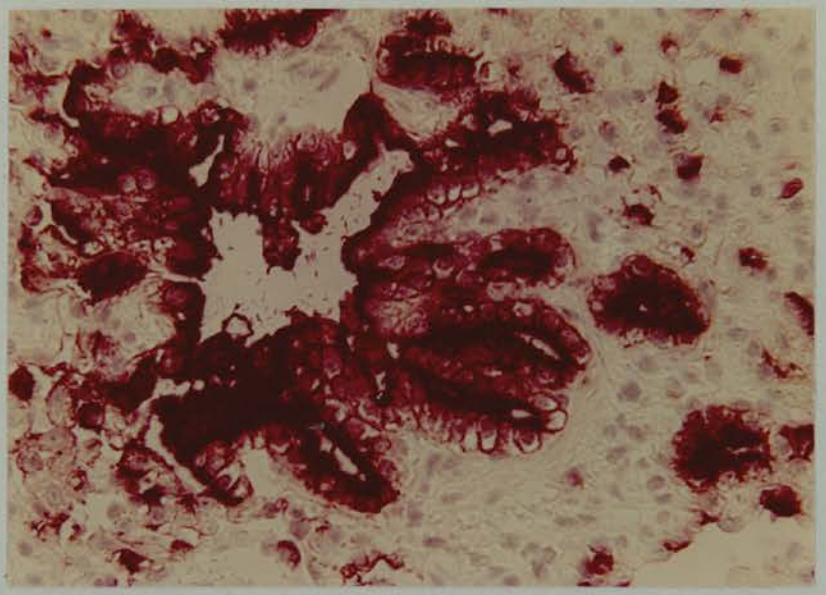

Fig.176 (Cat 4.) : Lung, 96 hours after operation. Alkaline phosphatase. Positive reaction in the bronchial epithelium in the periphery of the wound. x 350.

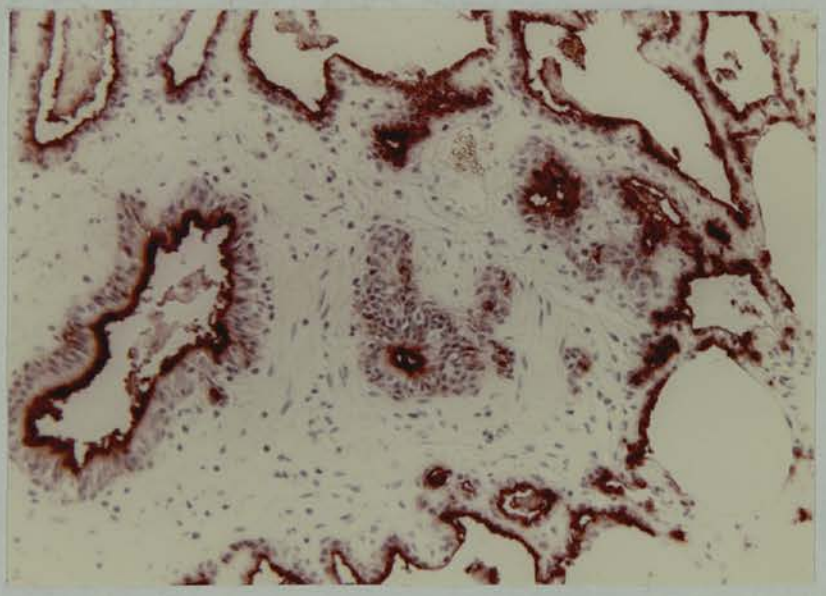

Fig.177 (Cat 7.) : Lung, 8 days after operation. Alkaline phosphatase reaction. Strongly positive in the bronchial epithelium at the margin of the wound and in the cubical cells of the alveolar walls adjacent to the fibrous tissue. x 168.

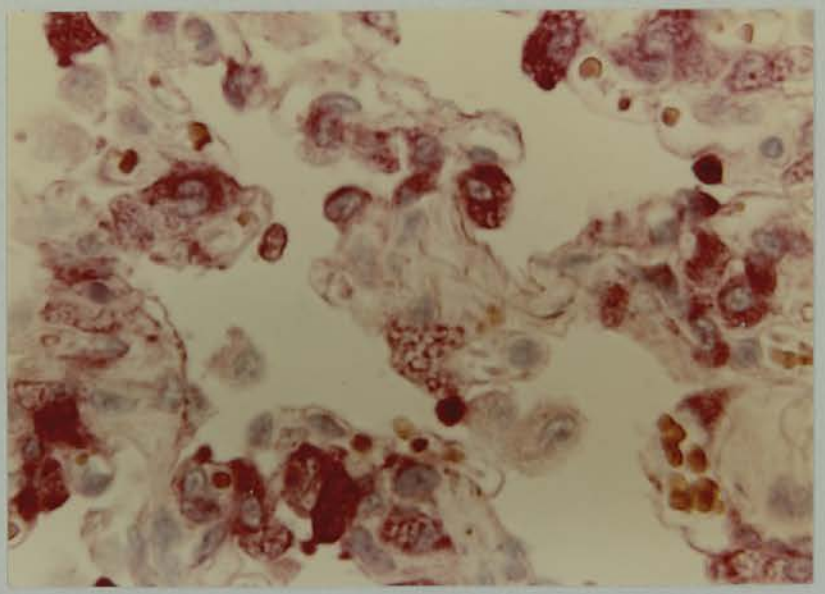

Fig.178 (Cat 4.) : Lung, 96 hours after operation. Positive esterase reaction in the cubical lining cells of the alveoli. x 550.

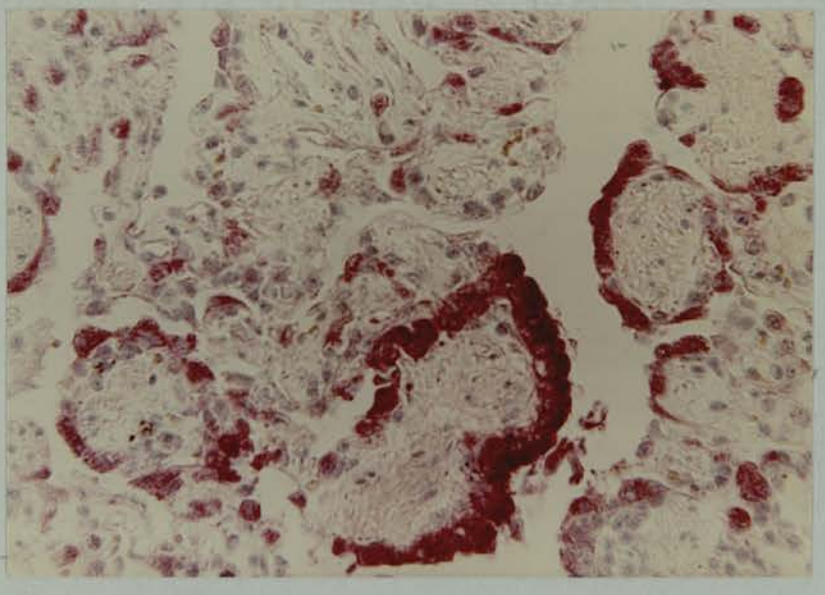

Fig.179 (Cat 6.) : Lung, 6 days after operation. Positive esterase reaction in the swollen alveolar lining cells. x 350.

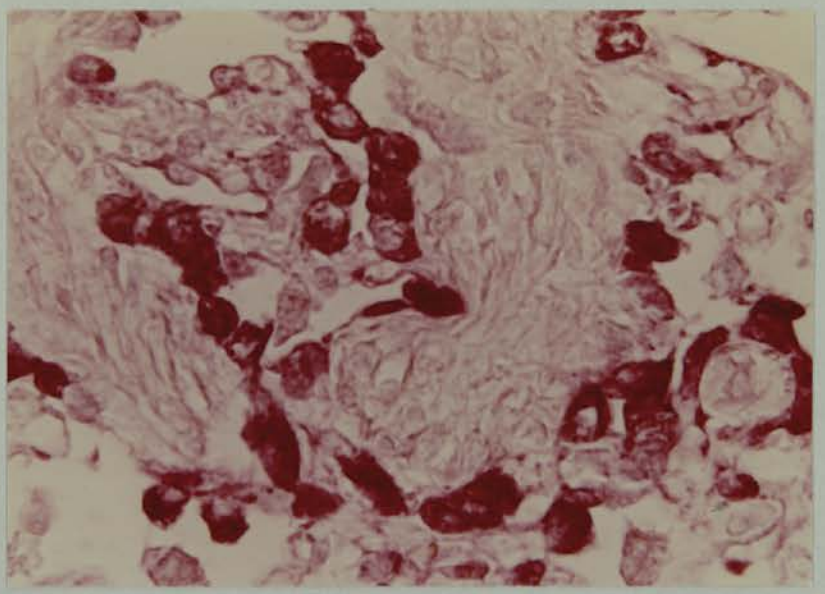

Fig.180 (Cat 11.) : Lung, 12 days after operation. Positive esterase reaction in the lining cells of new alveoli. x 650.

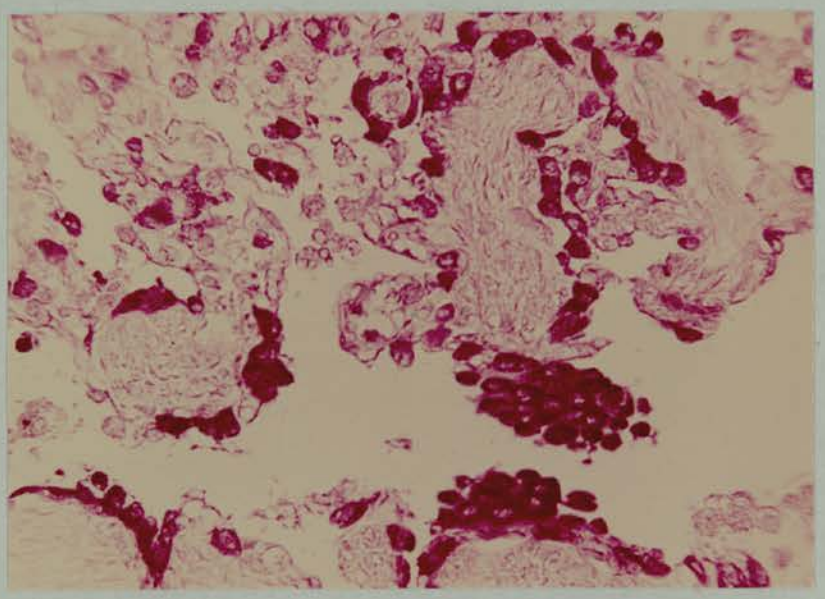

Fig.181 (Cat 11.) : Lung, 12 days after operation. Esterase reaction in the proliferating alveolar cells. x 325.

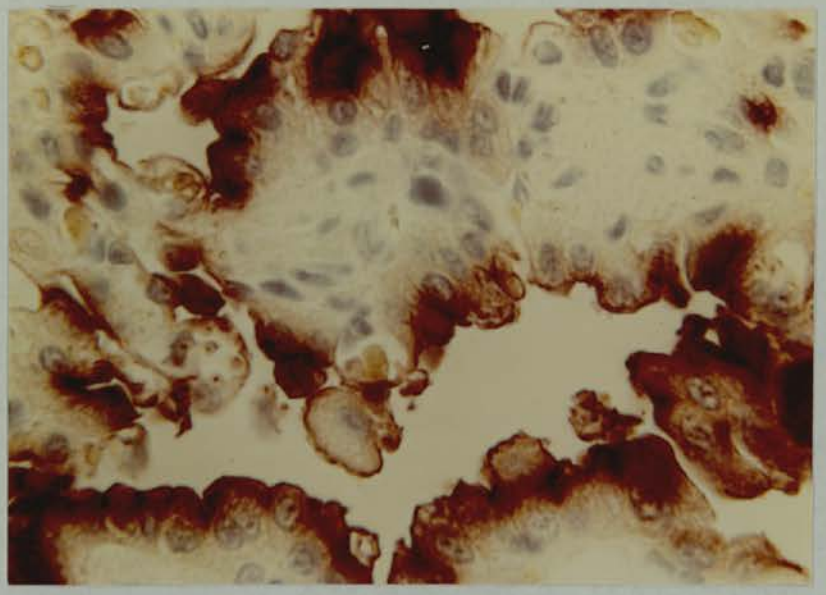

Fig.182 (Cat 5.) : Lung, 4 days after operation. Positive alkaline phosphatase reaction in the large macrophage cells(right) and in the swollen alveolar lining cells. x 550.

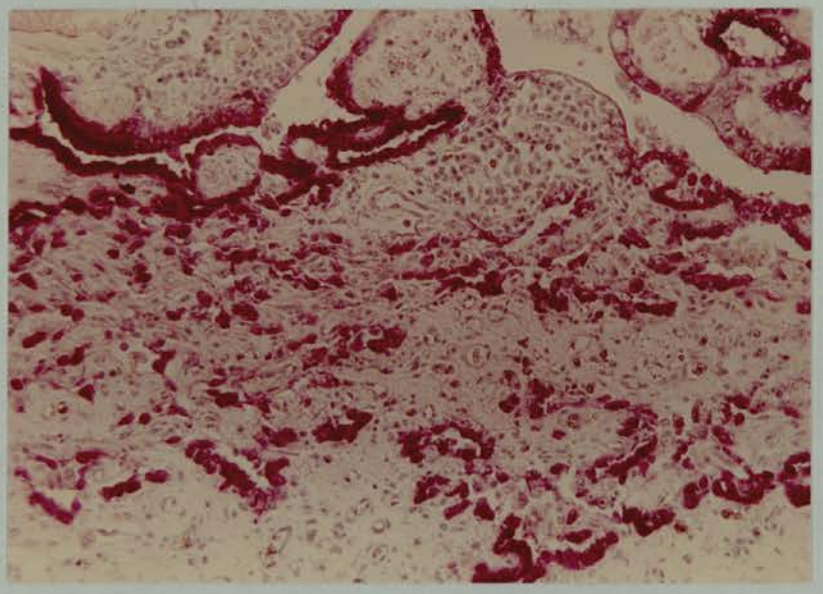

Fig.183 (Cat 12.) : Lung, 20 days after operation. Positive esterase reaction in the lining cells of newly-formed bronchi and alveoli. x 168.

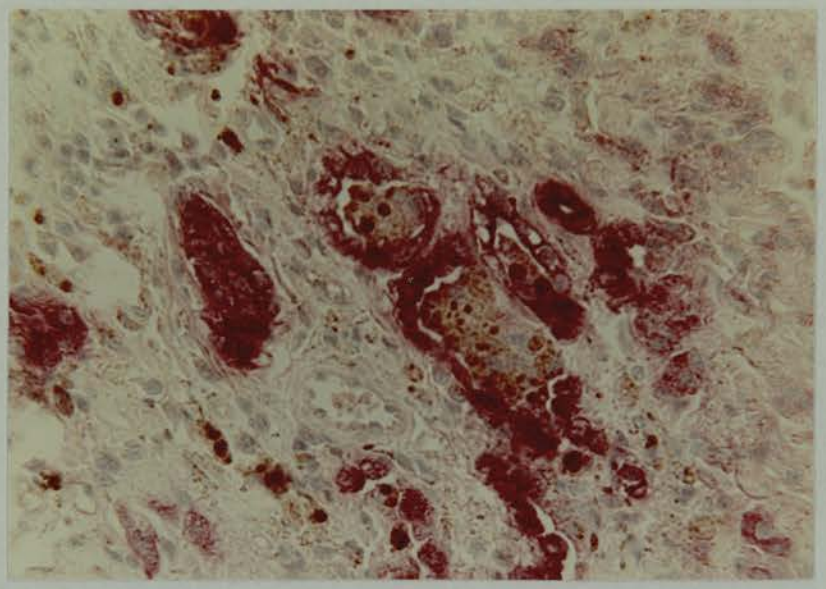

Fig.184 (Cat 13.) : Lung, 35 days after operation. Esterase reaction in the lining cells of bronchial buds. x 350.

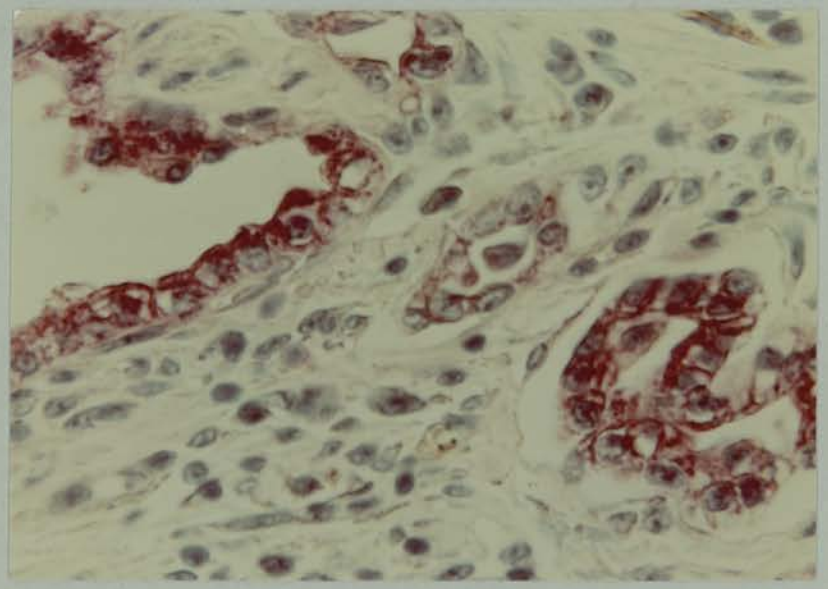

Fig.185 (Cat 15.) : Lung, 90 days after operation. The lining cells of bronchial buds showing positive esterase reaction. x 550.

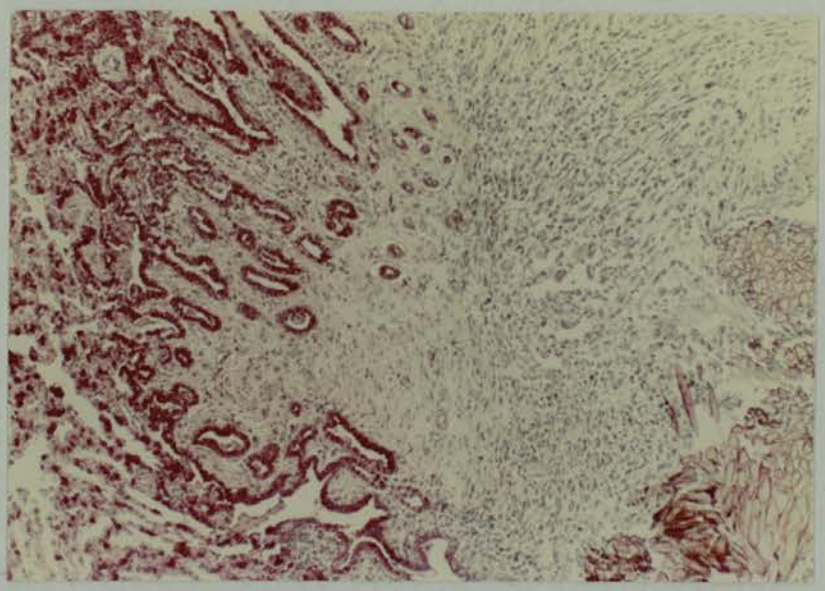

Fig.186 (Cat 18.) : Lung, 130 days after operation. Intense positive esterase reaction in the lining cells of developing bronchi and alveoli near the wound margin. x 90.

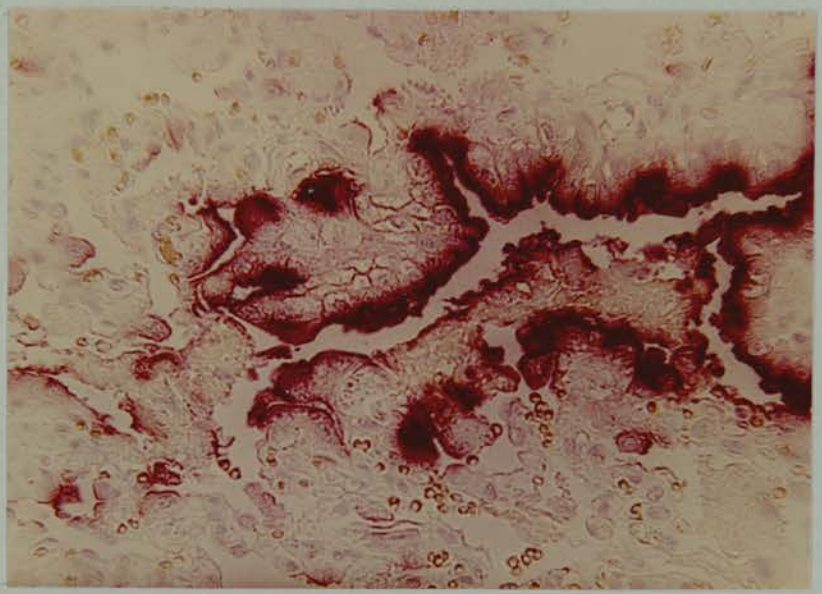

Fig.187 (Cat 12.) : Lung, 20 days after operation. Alkaline phosphatase reaction in the lining epithelium of bronchial buds. x 350.

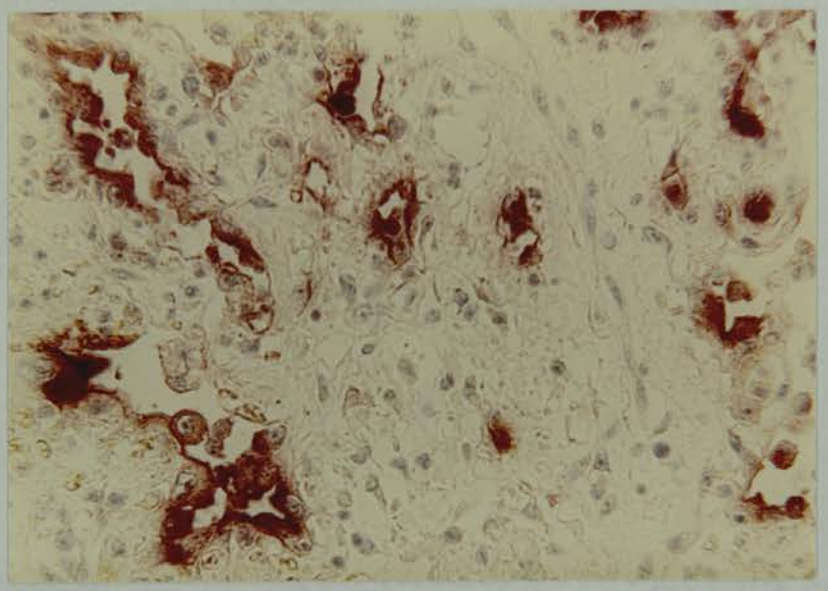

Fig.188 (Cat 6.) : Lung, 6 days after operation, showing mild alkaline phosphatase reaction in the cells of bronchial buds in an organised area. x 350.

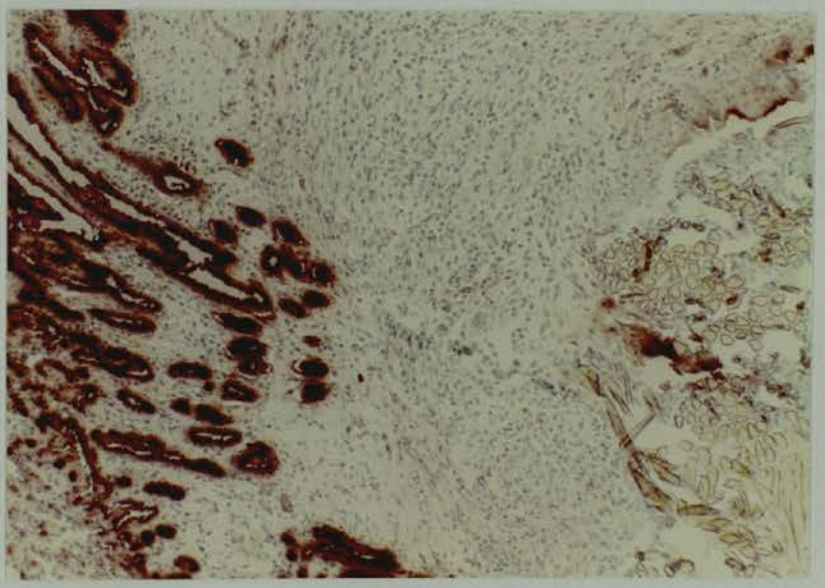

Fig.189 (Cat 18.) : Lung, 130 days after operation. Intense positive reaction for alkaline phosphatase in the cells of the bronchial buds penetrating a fibrous area. x 90.

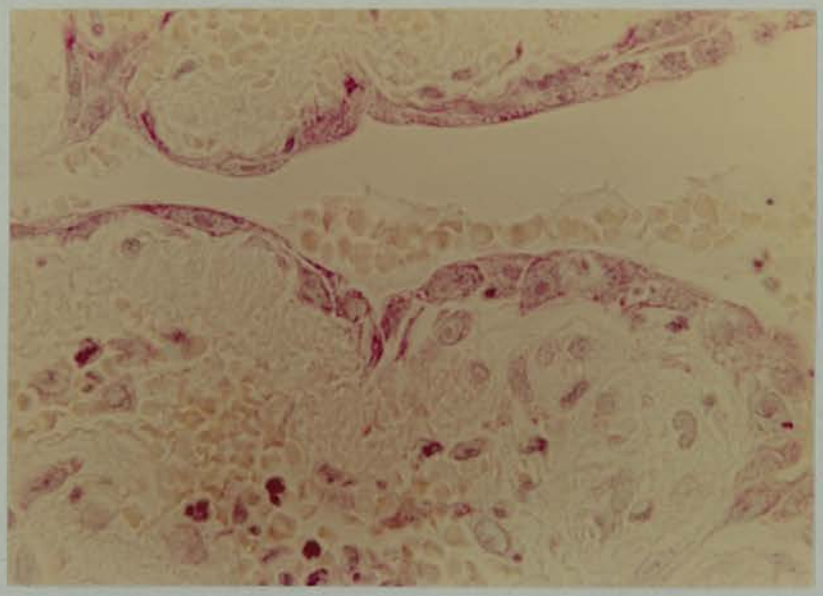

Fig.190 (Cat 4.) : Lung, 96 hours after operation. Mild reactions for R.N.A. & D.N.A. in the flattened, elongated epithelium of a bronchus. x 550.

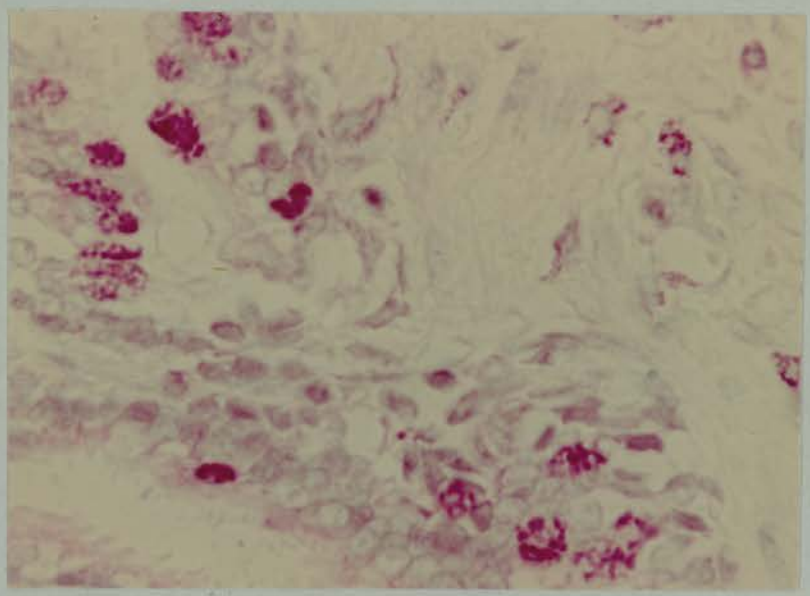

Fig.191 (Cat 8.), : Lung, 11 days after operation. Positive R.N.A. and D.N.A. reactions in the proliferating epithelium of a bronchus. x 650.

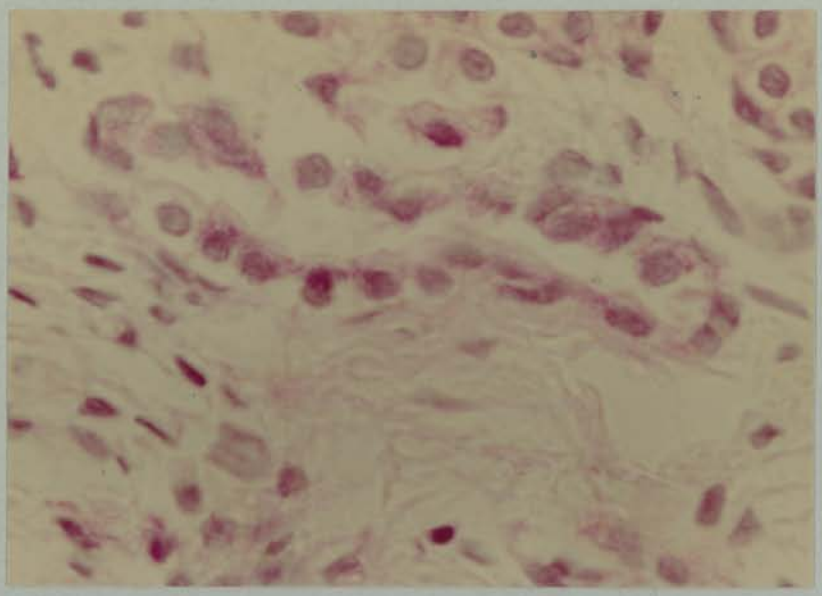

Fig.192 (Cat 11.) : Lung, 12 days after operation. Cubical lining cells of bronchial buds showing positive reactions for R.N.A. and D.N.A. x 1000.

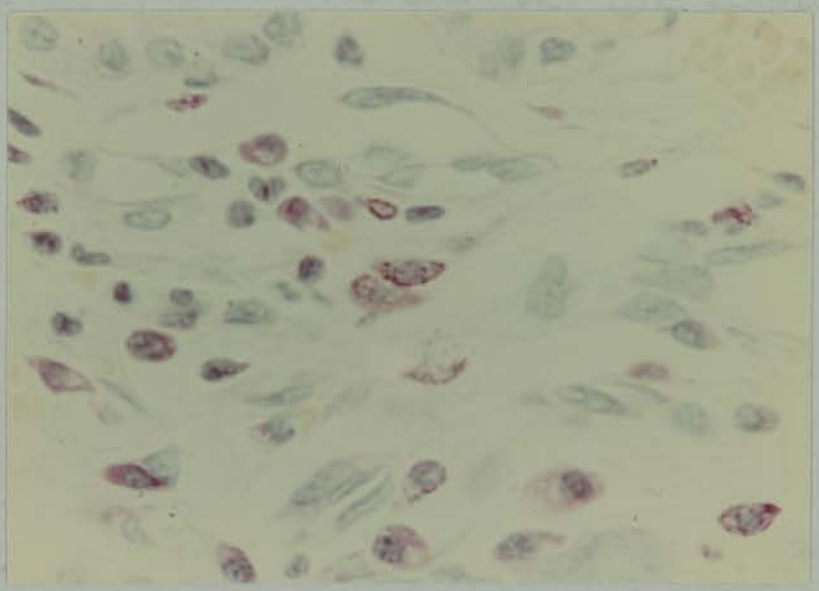

Fig.193 (Cat 14.) : Lung, 90 days after operation. Some scattered cells in the organised area giving positive staining reactions for R.N.A. and D.N.A. x 650.

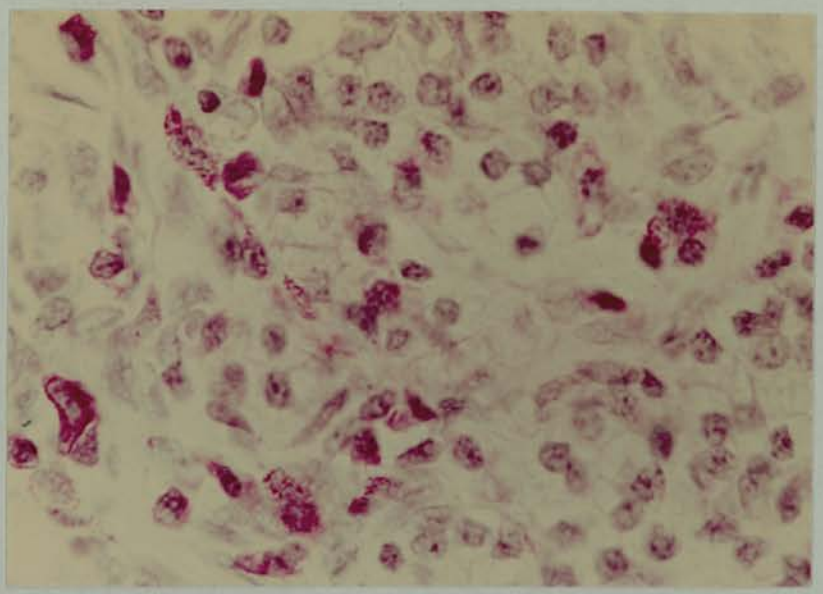

Fig.194 (Cat 18.) : Lung, 130 days after operation. Some cells in the organised area of the wound showing R.N.A. and D.N.A. activities. x 650.

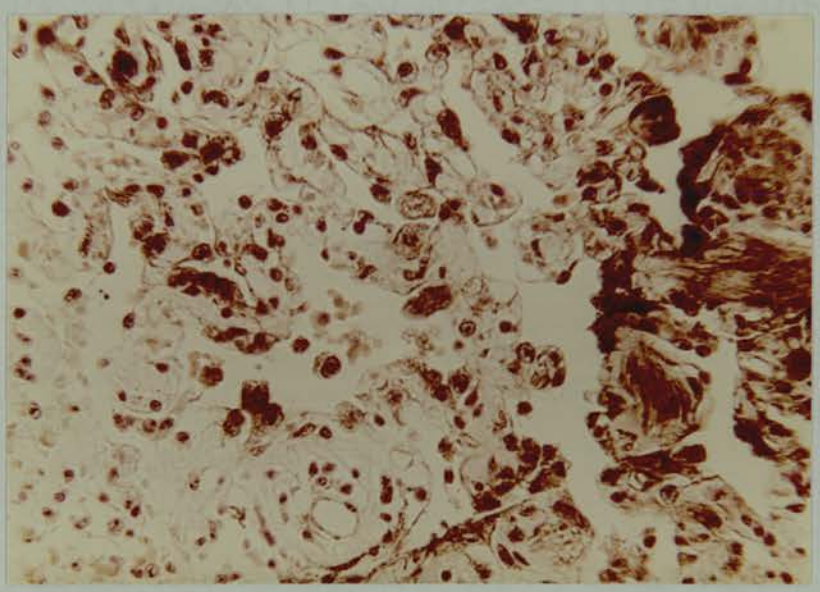

Fig.195 (Cat 6.) : Lung, 6 days after operation. The proliferating bronchial epithelium shows positive acid phosphatase reaction. x 350.

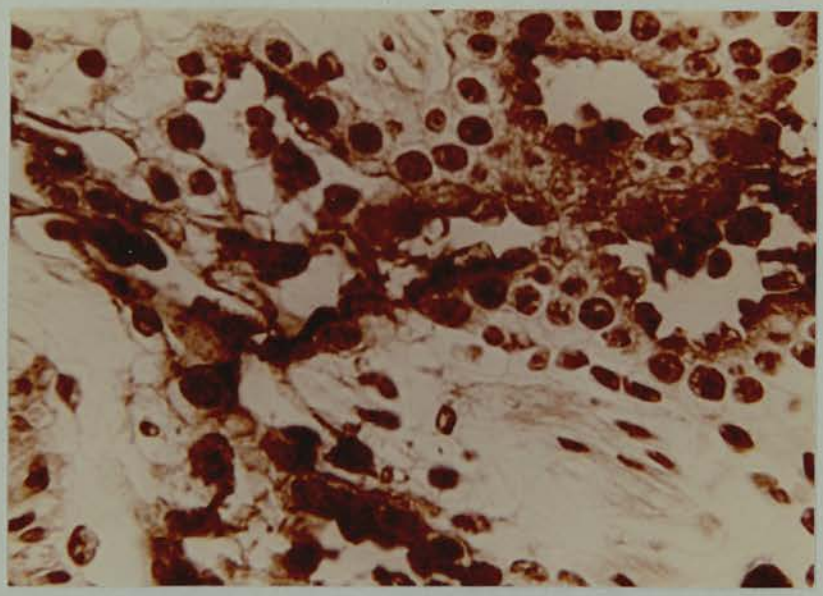

Fig.196 (Cat 11.) : Lung, 12 days after operation, showing acid phosphatase reaction in the cubical lining cells of bronchial buds and new alveoli. x 650.

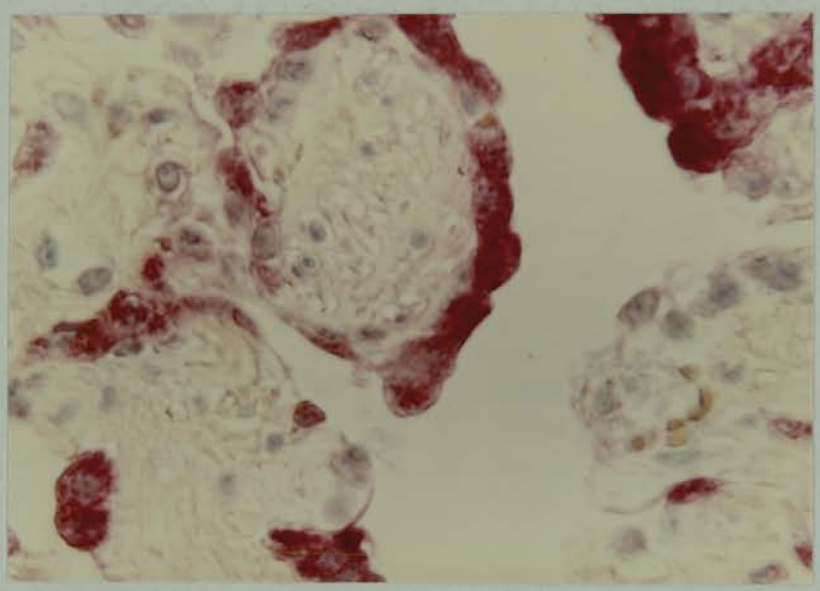

Fig.197 (Cat 6.) : Lung, 6 days after operation. Esterase reaction - positive in the cubical lining cells of alveoli. x 650.

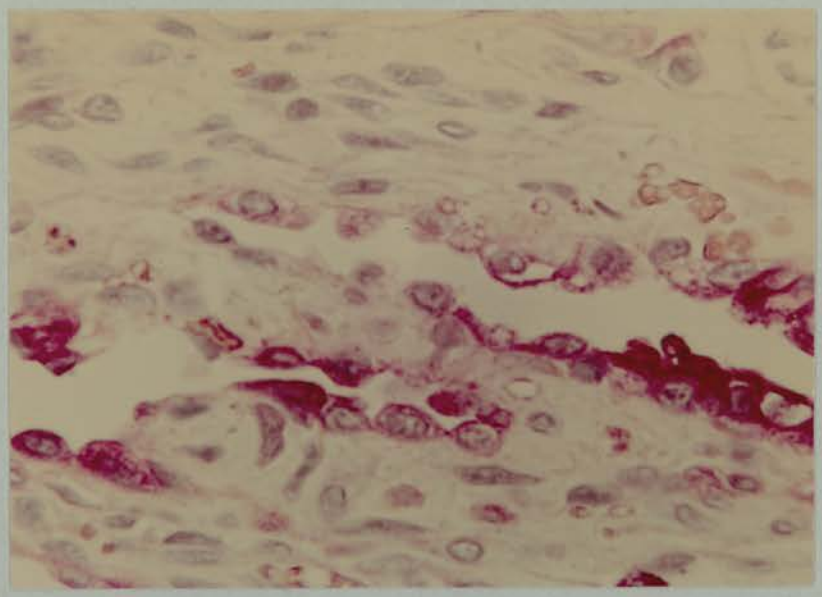

Fig.198 (Cat 12.) : Esterase reaction in the cubical cells of bronchial bud. x 550.

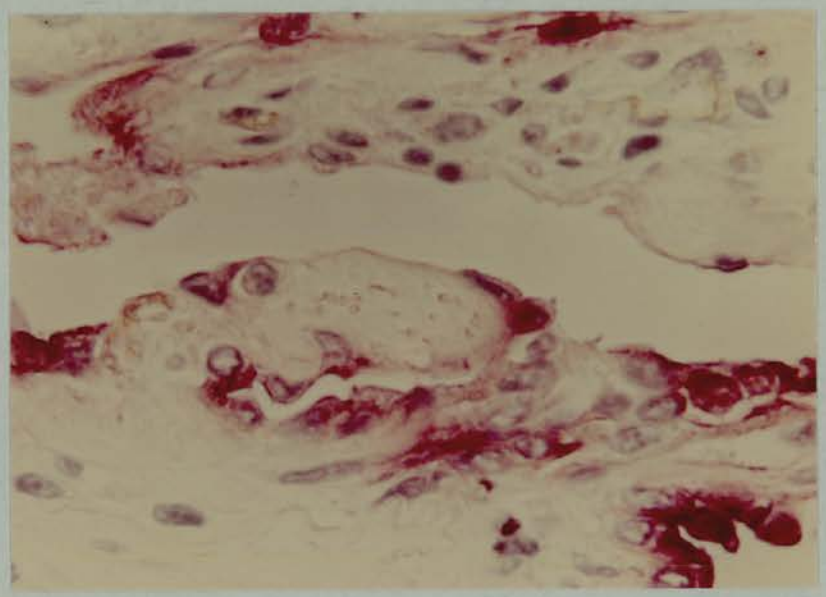

Fig.199 (Cat 11.) : Lung, 12 days after operation, showing alkaline phosphatase reaction - positive only in the cubical cells. x 650.

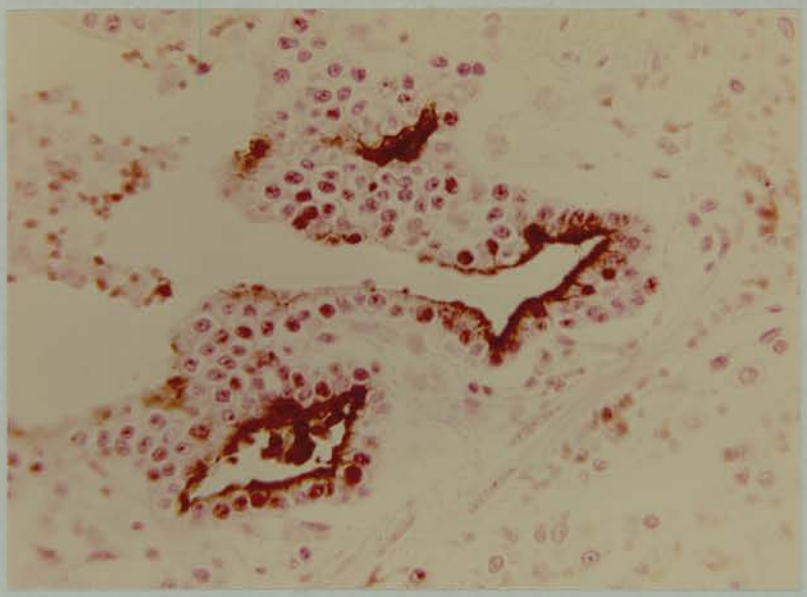

Fig.200 (Cat 14.) : Lung, 90 days after operation, showing positive acid phosphatase reaction in cubical cells of bronchial buds. x 168.

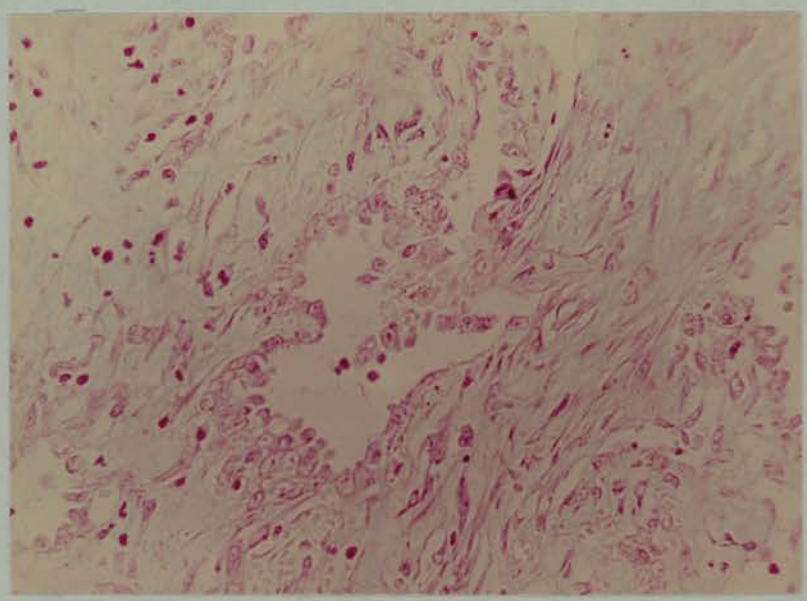

Fig.201 (Cat 7.) : Lung, 8 days after operation. Positive alcian blue reaction in an area of fibroblastic reaction but negative in the lining cells of bronchial buds and new alveoli. x 325.

SECTION II

(PART III)

AN AUTORADIOGRAPHIC STUDY OF THE UPTAKE OF $Na_2{}^{35}SO_4$ BY THE PULMONARY EPITHELIUM IN EXPERIMENTAL WOUNDS OF LUNG IN CATS

AN AUTORADIOGRAPHIC STUDY OF THE UPTAKE OF $Na_2{}^{35}SO_4$ BY THE PULMONARY EPITHELIUM IN EXPERIMENTAL HEALING WOUNDS OF LUNG IN CATS

INTRODUCTION

Not much has previously been reported about the uptake by the terminal units of the respiratory tree of ^{35}S from injected sulphate, and no relevant paper has been found on the same work, in lung, during the reparative phase of experimental wounds. Odeblad and Bostrom (1952), Bostrom and Obeblad (1953) and Bostrom (1953-54) did some works on the study of the uptake of ^{35}S by the tracheal and respiratory epithelium and tracheal cartilage of normal lung in rats and rabbits. Jennings (1958) studied the uptake of ^{35}S by the mucous secreting epithelium of the trachea of normal lung and gall-bladder in rat, rabbit, guinea-pig, cat and mice. These works were designed mainly to detect the presence of mucopolysaccahrides in the mucous-secreting cells of various organs by labelled isotopes, under normal condition. For this reason it has seemed worth while to record in this section of my thesis, the findings of the uptake by the pulmonary parenchyma of ^{35}S from/

from injected Na-sulphate during the reparative phase of experimental wounds of lung in cats. It is also hoped that this investigation may help to record some additional findings which may further contribute to the study of regeneration of lung tissue in experimental wounds in animal.

The distribution of sulphate, labelled with ^{35}S was determined by autoradiographs in which the position of concentrated radioactivity due to ^{35}S in the tissues at the time animal is killed is shown by a deposit of black particles of metallic silver in the photographic emulsion over the area, with some scattering around the site of maximum concentration.

EXPERIMENTAL PROCEDURE

Ten healthy adult cats were used. The animals were anaesthetised by intratracheal ether and oxygen under positive pressure anaesthesia and thoracotomy was performed as described in the previous section (Part III, section I) and comparable lung wounds were prepared in the left lower lobe.

Radioactive isotope was injected intraperitoneally into each animal before killing; the/

the interval between the injection and killing being 15 hours in each case. The isotope used was carrier-free sulphate ($Na_2{}^{35}SO_4$) in sterilised isotonic saline. (Obtained from the Radiochemical Centre of the United Kingdom Atomic Energy Authority through the kindness of the Medical Physics Unit of Edinburgh University). Radioactive sulphate iron has a half-life of 87.1 days and releases 'beta' particles of 0.167 MeV energy. It was injected at pH 7 in an average dose of 4uC per gramme of body weight. This dose was based on that recommended by Professor Montgomery personally, and was approximately 3500uC - 4000uC for each cat. The animals were killed by intra-peritoneal injection of nembutal (10 cc) at intervals of from 48 hours to 30 days after operation.

PREPARATION OF MICROSCOPICAL SECTIONS FOR AUTORADIOGRAPHY.

After death the heart and the lungs of the animal were removed en masse by opening the thorax in the midline. The lungs were expanded after removal with intratracheal infusion of formol-saline, fixed for 48 hours in a large volume of this fluid. Small blocks were taken/

taken after 48 hours of fixation from the area of the wound. These were fixed for another 24 hours in 10 per cent neutral formol-saline after trimming them to suitable size. They were then dehydrated in usual manner, impregnated with paraffin and embedded in vacuo. Sections were cut at 5u thickness and mounted on glass slides which were previously 'subbed' in a 'subbing solution' of potassium bichromate and sulphuric acid to ensure good wet-adhesion of the emulsion film when autoradiographs were processed.

Mounted slides were dewaxed with xylol, taken through water and one of each series was stained with haemotoxylin and eosin for routine examination. For identification of mucopoly-saccharide, alcian blue and P.A.S. (periodic-acid-fuschin-sulphorous acid) stains were used.

The stained sections were exposed to stripping film emulsion, in a dark room, for the production of autoradiographs by attaching the emulsion to the section for permanent contact. The exposed slides were dried, placed in a slide-box and stored in a cold light-tight steel cabinet in the dark room for the required period of exposure of the film. As a preliminary test

a/

a number of sections covered with emulsion film were exposed in a series for a period of from 1 week - 12 weeks and developed and fixed. Optimum concentration of radioactivity was obtained, in this series of sections, about 6 - 8 weeks after exposure. As a rule, 7 weeks after exposure the emulsion film, attached to the sections, was developed subsequently, in all the cases, in a weakly alkaline Kodak 'Dolmi' developer (D170), rinsed in water to neutralise the excess developer and then fixed in a solution of Kodak-acid-fixer powder at 20°C. The preparations were next washed thoroughly in running water, dried, dehydrated, cleared and mounted in DPX for microscopical examination. Few sections of normal lung tissue were prepared and processed in similar way for autoradiography.

OBSERVATIONS AND RESULTS

The results are given in Tables I, II and III, and are examplified in Figs. 202 - 215.

TABLE/

TABLE I

Sulphate fixation in the parenchyma of normal lung

Animal	Tissue	Site	Amount of 35-S	Mucopoly-saccharide		Notes
				PAS	AB	
Cat 26	Trachea and Bronchi	Surface Epithelium	+++	+	+	High concentration of ^{35}S in the supranuclear region of goblet cells.
		Submucosal mucous gland	+++	+	+	High concentration of ^{35}S in the intracellular mucin mass, in the free border of the cells.
		Cartilage plate	++	+	-	Diffuse uptake.
		Lumen of bronchi	+	+	-	Black grains of metallic silver are visible when mucin was present in the lumen.
	Terminal bronchiole	Surface epithelium	(+)	(+)	(+)	Presence of radioactive material was doubtful.
	Alveoli	Lining cells	(+)	-	-	One or two grains of black metallic silver were visible.

TABLE II

Sulphate fixation in the parenchyma of healing lungs

Animal	Wound	Tissue	Site	Amount	Mucopoly-saccharide		Notes
					PAS	AB	
Cat 2	48 hours	Trachea	Surface Epithelium	+++	+	+	High concentration of ^{35}S in the supranuclear region of goblet cells.
			Submucosal mucous glands	+++	+	+	High concentration of ^{35}S in the intracellular mucin mass.
			Cartilage plate	++	+	-	Diffuse uptake.
		Bronchi, (near the wound)	Proliferating bronchial epithelium	++++	+	-	Very high concentration of ^{35}S in the main intracellular mucin mass of goblet cells.
			Mucosal gland	++++	+	+	Very high concentration of ^{35}S in the intracellular mucin mass.
			Lumen	++	-	-	Positive only when there was enough mucin mass present.
			Cartilage plate	++	+	-	Diffuse uptake.
		Bronchiole (near the wound)	Surface epithelium	+++	(+)	(+)	Radioactivity was definitely present in the surface epithelium in high concentration.
		Alveoli, (near the wound)	Lining cell	(+)	(+)	-	Doubtful, only one or two grains of black metallic silverpresent on the alveolar wall.
		Organising area	Area of fibroblastic reaction	++	+	+	Presence of 35S could be seen in association with fibroblast cells.

TABLE III

Sulphate fixation in the pulmonary parenchyma of healing lung

Animal	Wound	Tissue	Site	Amount of ^{35}S	Mucopoly-saccharide PAS	Mucopoly-saccharide AB	Notes
Cat Nos. 22 23 24 25 26 27 28 29 30	72 hours to 30 days	Trachea and bronchi, (near the wound) and bronchioles (near the wound margins)	Similar sites as in the case of the cat killed 48 hours afterop-eration	Same as that of 48 hours old wound	Same positive reaction as that of 48 hours old wound		Similar concentration of radio-activity was noticed as seen in 48 hours old wound.
		Regenerat-ing areas	Bronchial buds	++	+	-	Radioactivity was seen in the cubical cells lining the bronchial buds.
			New al-veoli	+	(+)	-	Cubical cells lining the new al-veoli exhibited some radioactivity.
		Organised areas	Fibroblast cells	++	+	+	Fibroblast cells showed moderate degree of radioactivity.

++++ = Very large amount of ^{35}S.
+++ = Large amount of ^{35}S.
++ = Moderate amount of ^{35}S.
+ = Only a trace
(+) = Doubtful.
- = No radioactivity.

The results shown in Table I, II and III require a little elucidation at few points :

It was observed that the epithelium of the trachea of both normal and the healing lungs showed equal concentration of ^{35}S (Fig. 202), but the proliferating epithelium of bronchi, near the wound margins of the operated lung showed higher concentrations of radioactivity in the goblet cells (Figs. 203 - 205) than in the goblet cells of normal bronchial epithelium, (Fig. 206, 207). The uptake of ^{35}S by the scanty lining cells of normal alveolar walls could not be detected, though on microscopical examination of sections of normal lung one or two grains of black metallic silver could often be seen along the line of the alveolar wall. (Fig. 208.) On the other hand, uptake of ^{35}S by the cubical cells of bronchial buds and new alveoli was observed in the regnerating area. (Figs. 209, 210, 211, 212). Organising areas of the wound showed scattered presence of ^{35}S specially in close association with the fibroblast cells. (Fig. 213). To exclude possibility of artefact or any dispersion of grains of metallic silver in the above sites, several sections of tissue from the same paraffin block were/

were prepared and similarly processed for examination. All these sections showed similar appearances and concentration of ^{35}S in similar fields of the regenerated area of the wound.

Free mucous on the surface of the mucosa and in the lumen of bronchi or bronchioles, when present, was radioactive in all specimens. (Figs. 214). Bronchial cartilage plate of normal and of healing lungs showed diffuse concentration of ^{35}S. (Fig. 215).

DISCUSSION

Much work has been done recently on the uptake of ^{35}S given as sulphate ions to animals in normal condition. The results help to explain the apparent specificity of the autoradiographic methods for mucopolysaccharides. When ^{35}S in the form of sulphate ion is given to animals it may follow several pathways. Most of it very quickly excreted as inorganic and etheral sulphate; a portion is retained in sulphated mucopolysaccharides and a trace is found in the sulphur-containing amino-acid cystine. These findings were based on chemical extraction studies of Curan and Kennedy (1955). The fraction of ^{35}S-sulphate retained by various tissues/

tissues has been shown by chemical extraction to be present as esterified form such as chondroitin sulphate - in skeletal cartilage and intestinal tract (Dziewiatkowski, 1951, 1953, 1954); in skin (Bostrom and Gardell, 1953); in connective tissue ground substance and collagen (Layton, 1951); in aorta, spleen, kidney, tibia, red-marrow, heart and skeletal muscles of chick embryo (Layton, 1952); in the healing wound in the hen (Layton, 1950, 1952) and in fibroblasts (Curran and Kennedy). The last team of workers confirmed by autoradiographic techniques the cytological distribution of the ion retained within the above tissues and showed its invariable association with mucopolysaccharides. They demonstrated that the highest activities of ^{35}S were within cells which formed mucopolysaccharide, such as intestinal goblet cells, cartilage cells and mast cells.

In most of the tissues (organs and tissues belonging to the gastro-intestinal tract and cardiovascular system, some parenchymatous organs, the eye and the trachea) where mucopolysaccharides are known to be present a considerable uptake of ^{35}S has been demonstrated by means of autoradiography/

autoradiography in rats and rabbits by Odeblad and Bostrom. Jennings and Floery (1956) have shown the incorporation of sulphate into the mucous-forming epithelia of gastro-intestinal canal by identifying in autoradiographs the sites where ^{35}S, injected as $Na_2{}^{35}SO_4$, was localised.

Tracheal and respiratory epithelium of normal lung in rat and rabbit showed, in autoradiographs, a moderate degree of uptake of ^{35}S, injected as Na-sulphate (Odeblad and Bostrom; Bostrom, 1953-54). This uptake of ^{35}S by tracheal and respiratory epithelium is, according to Bostrom, possibly due to incorporation of the sulphate in the mucous. Jennings showed by autoradiographs that in the rat, rabbit, cat and guinea-pig both the goblet cells and the cells of the mucous glands of the trachea took up ^{35}S from injected Na-sulphate. Tracheal cartilage had been shown by autoradiography in rat and rabbit to take up ^{35}S by Odeblad and Bostrom and Bostrom and Odeblad. The results obtained from my present investigation has confirmed the observations of some of the above works so far as the tracheal structures and respiratory epithelium in normal lung are concerned. Further, my/

my observations are in concurrence with those of the above workers as regards to the close association of radioactive material with stainable mucopolysaccharides.

It is true that in most of the tissues where mucopolysaccharides are known to be present a considerable uptake of retained ^{35}S can be demonstrated in cat's lung. The goblet cells of surface epithelium and the mucous secreting cells of submucosal glands of the trachea, cartilage cells of both trachea and bronchi and the fibroblast cells of the organising areas of the wounds of lung in cat are stained with alcian blue and P.A.S. which indicated the presence of mucopolysaccahrides in these tissues. The stronger radioactivity of proliferating bronchial epithelium near the wound margins is, however, noteworthy.

The presence of radioactive material in the lining cubical cells of the regenerated bronchial buds and alveoli, however small in quantity, is a marked contrast to the findings of normal lung where the terminal bronchiolar epithelium and the scanty lining cell of alveolar walls are presumably devoid of radioactivity. The cubical cells of regenerated bronchial buds and alveoli give negative alcian blue staining reaction/

reaction.

The appearance of radioactivity in the epithelium of the regenerating bronchial buds and new alveoli suggests their direct descent from the epithelium of the parent stem which shows high concentration of ^{35}S near the margins of the wounds.

SUMMARY

The uptake of injected ^{35}S-labeled sodium sulphate by the respiratory epithelium during the reparative phase of experimental wounds of lung in cat has been studied by means of autoradiography.

In most of the sites, where mucopolysaccharides are known to be present, a considerable uptake of ^{35}S was demonstrated.

Proliferated bronchial epithelium near the wound margins showed very high concentration of radioactivity in the goblet cells. The presence of ^{35}S was observed also in the cubical cells lining the bronchial buds and new alveoli in the regenerating area of the lung.

The mucous cells in the gland and surface epithelium of the trachea and larger divisions of bronchi of both normal and healing lungs took/

took up ^{35}S and incorporated in their mucin. Cartilage cells of the trachea showed diffuse uptake of ^{35}S.

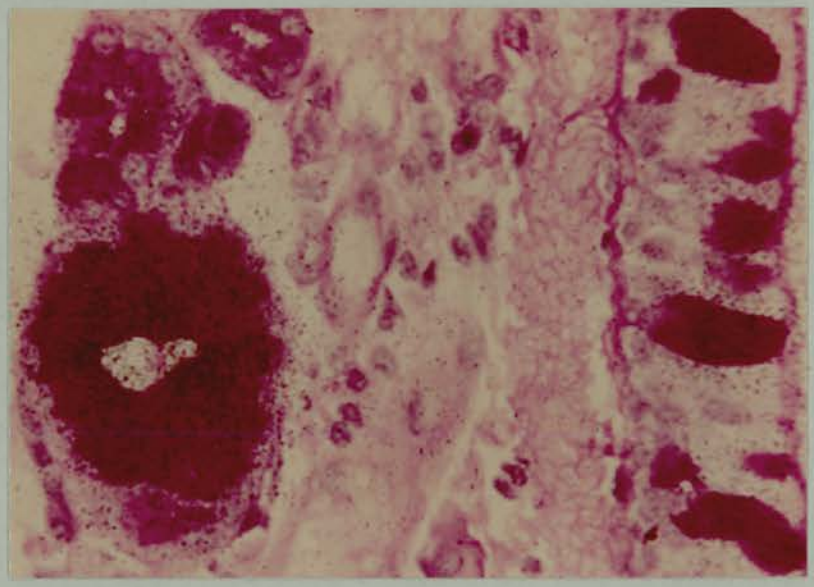

Fig.202 (Cat 26.) : Normal trachea. Deposit of radio-active sulphur in the goblet cells of the mucosa and in the cells of submucosal mucous-secreting glands. x 675. (P.A.S.)

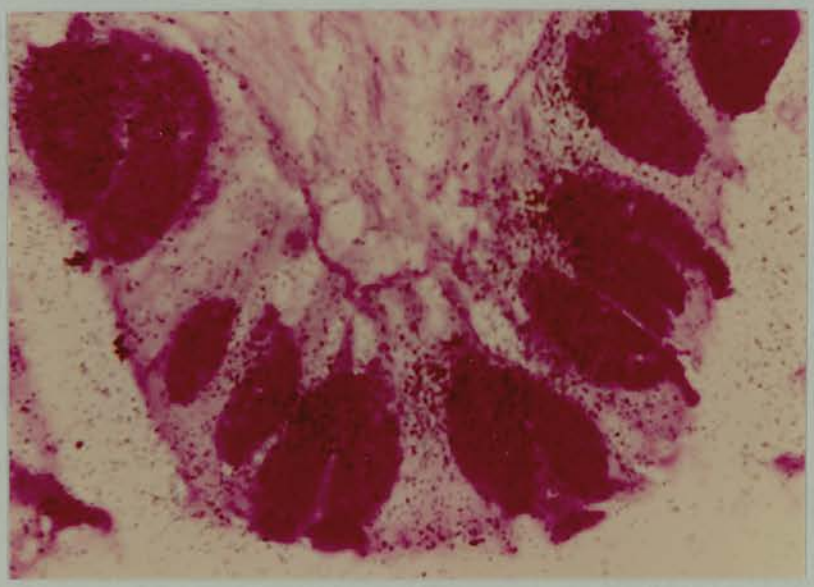

Fig.203 (Cat 27.) : 5 days after operation. Much higher concentration of radio-active sulphur in the epithelium of a bronchus near the wound margin. x 900. (P.A.S.)

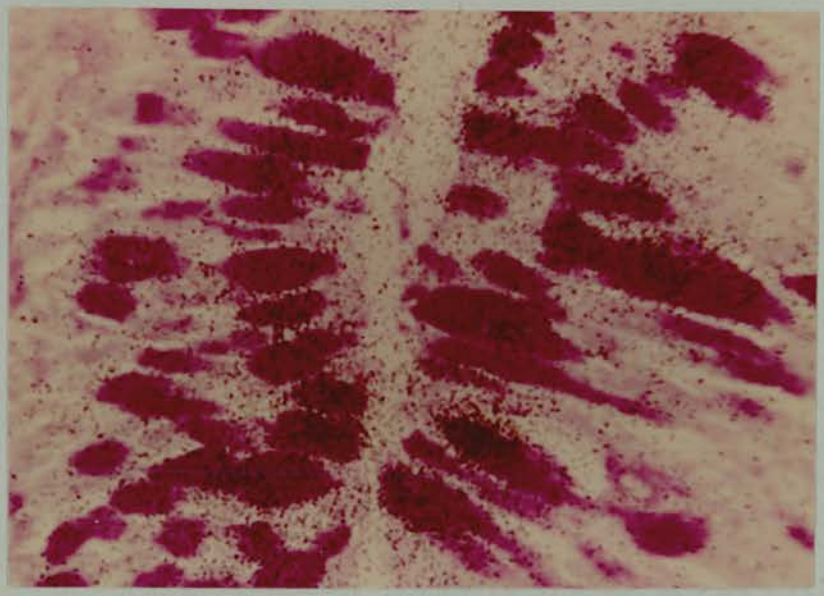

Fig.204 (Cat 28.) : Lung, 7 days after operation. The epithelium of a bronchus near the wound margin shows large amount of radio-active sulphur in the proliferating cells. x 750. (P.A.S.)

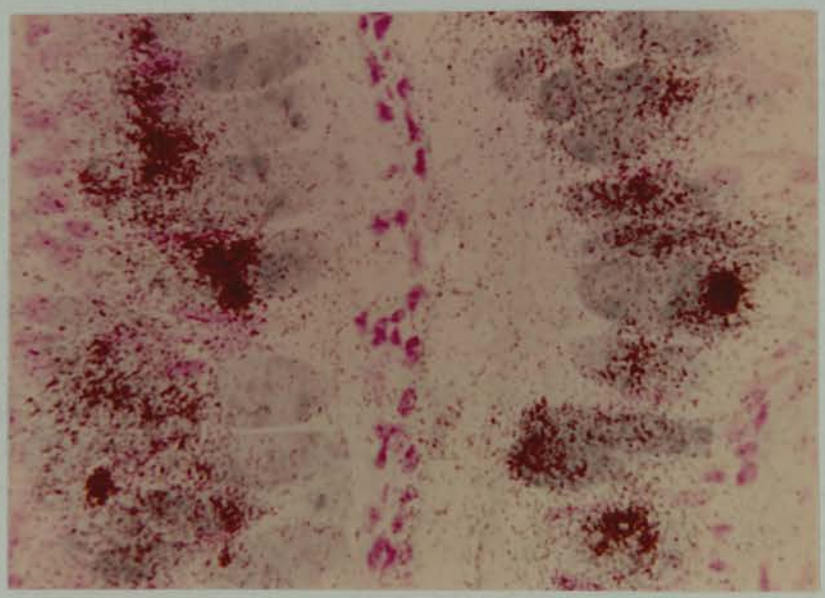

Fig.205 (Cat 30.) : Lung, 12 days after operation, showing deposit of large amount of radio-active sulphur in the proliferating cells of the epithelium. x 750. (Alcian blue.)

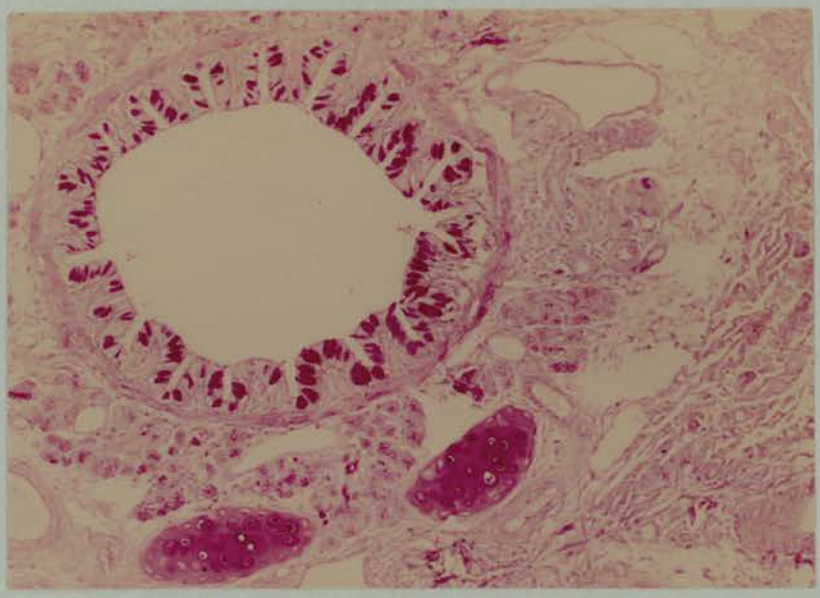

Fig.206 (Cat 26.) : Normal lung. The epithelium of the bronchus shows only moderate concentration of radio-active sulphur. x 90. (P.A.S.)

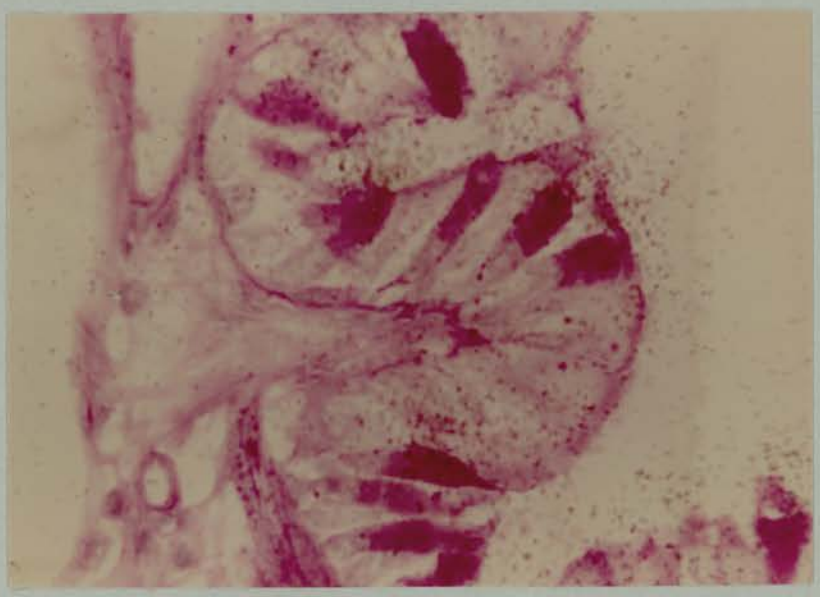

Fig.207 (Cat 26.) : High power of Fig.206, to show moderate concentration of radio-active sulphur in the lining cells of the epithelium. x 775. (P.A.S.)

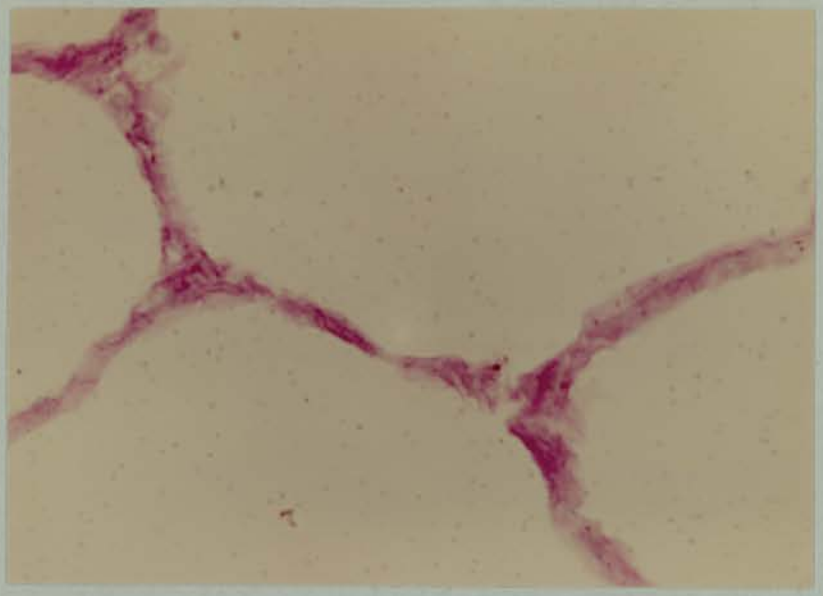

Fig.208 (Cat 26.) : Normal lung. The alveolar walls show only a few grains of metalic silver indicating slight radioactivity in the scanty lining cells of the alveoli. x 825. (P.A.S.)

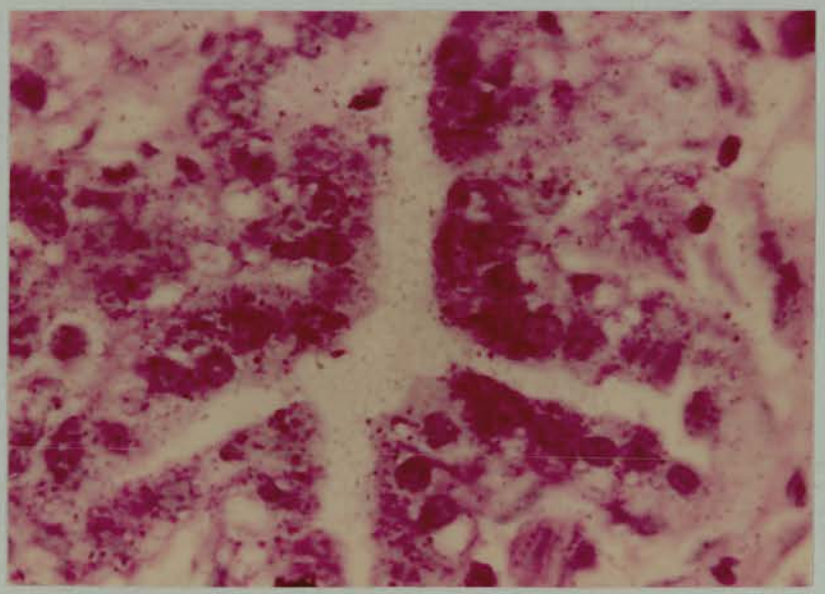

Fig.209 (Cat 30.) : Lung, 12 days after operation. The cubical lining cells of the bronchial buds show fairly moderate concentration of radio-active sulphur. x 900. (P.A.S.)

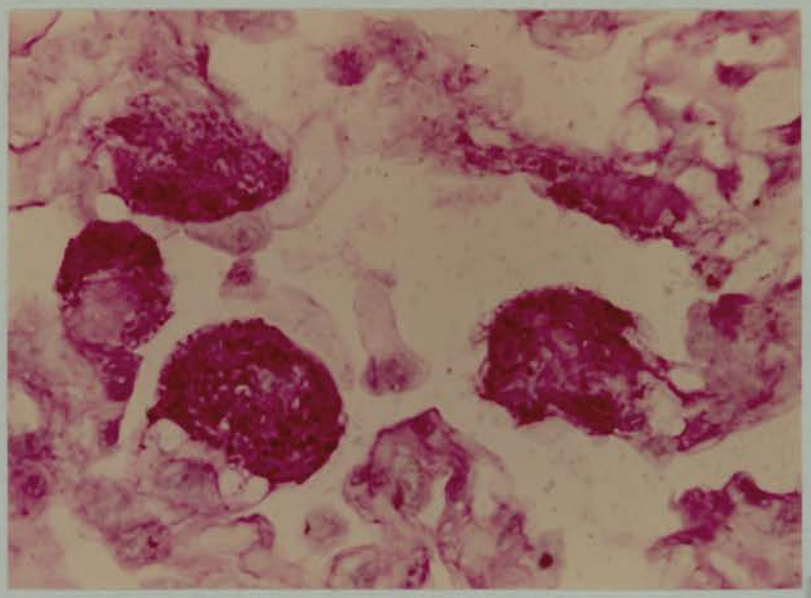

Fig.210 (Cat 27.) : Lung, 5 days after operation. The swollen alveolar lining cells from an area of regenerating lung, show deposit of radio-active sulphur in their cytoplasm. x 900. (P.A.S.)

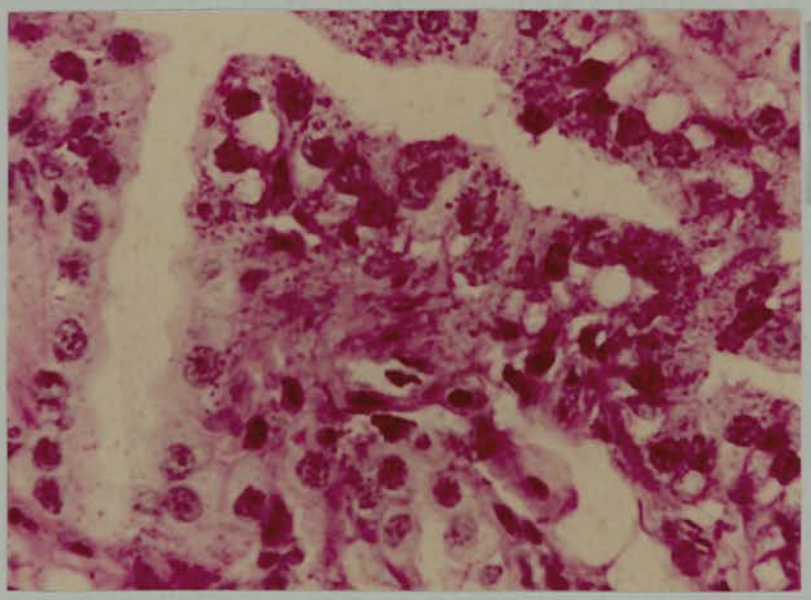

Fig.211 (Cat 30.) : Lung, 12 days after operation, showing presence of radioactivity in the cubical cells of developing bronchi. x 900. (P.A.S.)

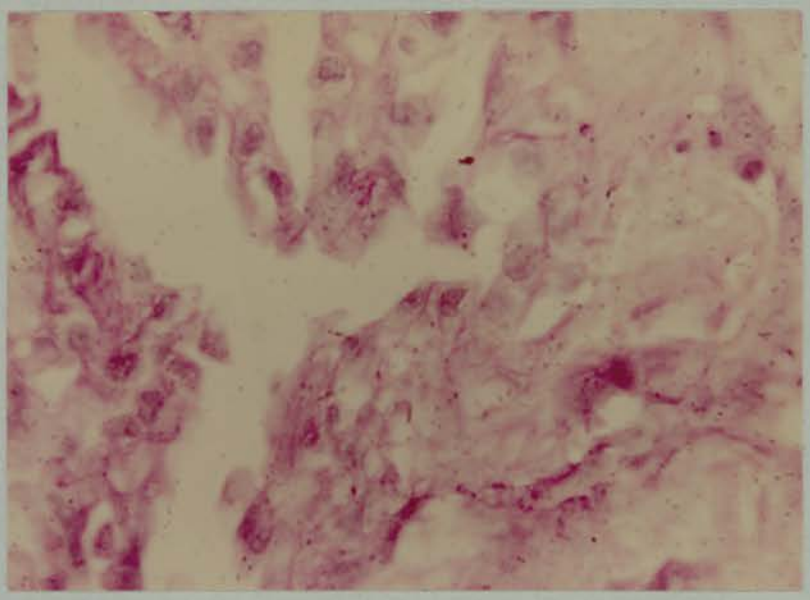

Fig.212 (Cat 31.) : Lung, 20 days after operation. Uptake of ^{35}S is evident by the lining cells of newly-formed alveoli in the regenerated lung. x 750. (P.A.S.)

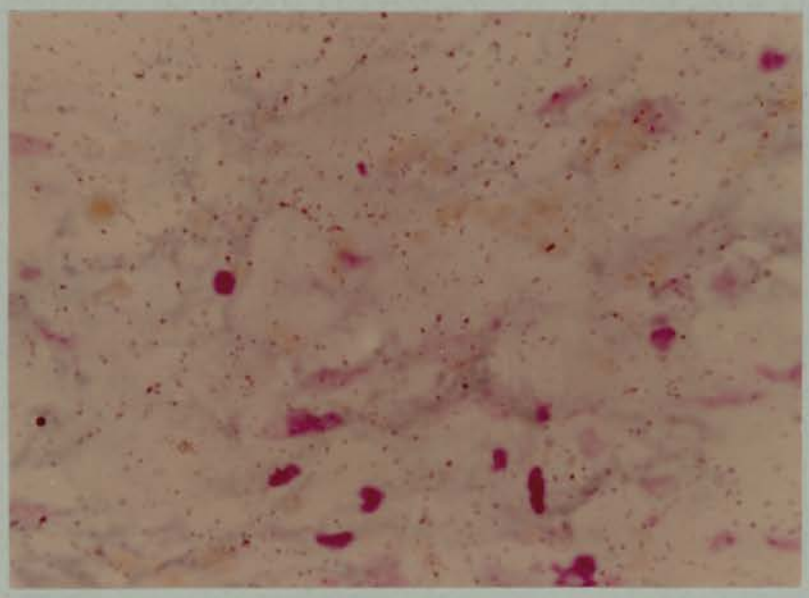

Fig.213 (Cat 27.) : Lung, 5 days after operation. Deposit of radio-active sulphur is seen in an area of fibroblastic reaction in association with fibroblast cells. x 775. (Alcian blue.)

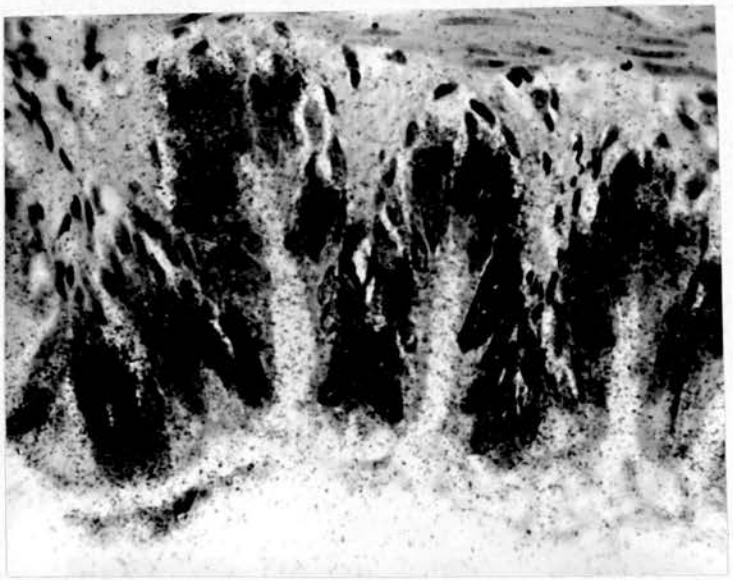

Fig.214 (Cat 29.) : Lung, 7 days after operation, showing radioactivity in the free mucous in the lumen of a bronchus. x 450. (H.E.)

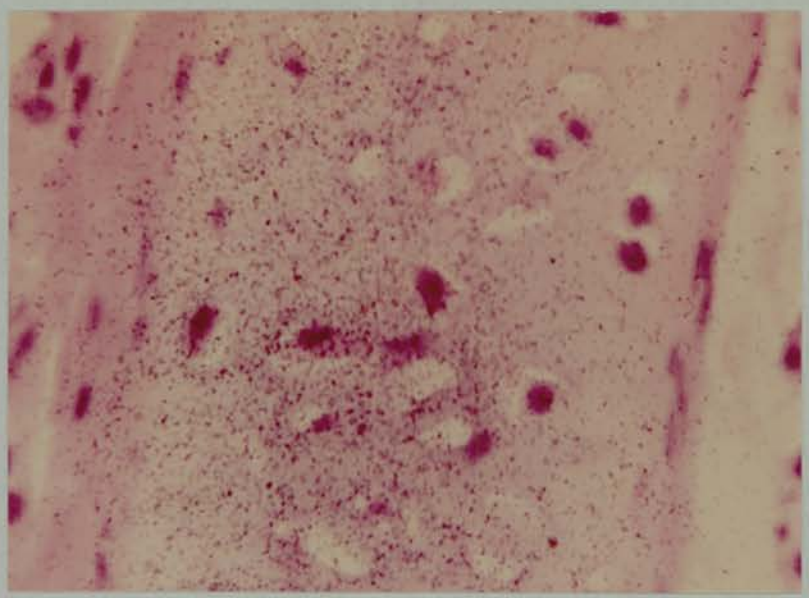

Fig.215 (Cat 25.) : Bronchial cartilage plate of both normal and healing lung shows diffuse concentration of ^{35}S in all specimens. x 750. (P.A.S.)

PART IV

STUDY OF THE REPARATIVE CHANGES OF EXPERIMENTAL PULMONARY INFARCTS IN CATS AND RABBITS.

STUDY ON THE REPARATIVE CHANGES OF EXPERIMENTAL PULMONARY INFARCTS IN CAT AND RABBIT.

INTRODUCTION

Since the days of Virchow (1856) a formidable number of experimental works had been done by many workers to produce pulmonary infarcts in animals by introducing various substances as emboli in the circulatory system. The experimental demonstration of embolism as the cause of pulmonary infarct came with the appearance of Cohnheim and Litten's article in 1875. They were able to produce pulmonary infarct in animal by introducing paraffin emboli into the pulmonary arterial circulation. Fujinami (1898), Orth (1897) and Zahn (1897) confirmed Cohnheim and Litten's observations. Fujinami used paraffin wax as emboli, injected into the jugular vein, so that the plugs would lodge not only in a large branch but also in several smaller branches of the pulmonary artery to shut off the collateral circulation. Orth used various chemical irritants with paraffin emboli and obtained fairly constant results. Zahn was the first to report that passive venous congestion increased/

increased the likelihood of pulmonary infarcts and, accordingly by binding the rabbit's thorax tightly two days afterthe emboli had lodged, was able to obtain typical infarcts in the lungs. Steinberg and Mundy (1936) reported that if a sufficient number of emboli were introduced, infarction of lung might occur. They showed this by introducing lead dust into the jugular vein in dogs and thought that earlier failures to secure infarcts embolically might be due to the introduction of insufficient number of particles.

Most of the recent editions of text-books on pathology accept the conclusion that an embolus in the absence of passive venous congestion, is incapable of producing a haemorrhagic infarct of lung. Experimentally a large number of different substances used by themselves have failed to produce pulmonary infarct. (Karsner and Ash, 1912 - 13 - turnip seeds in dog; Mann, 1917 - paraffin in mongrel; Gauss, 1924 - fat embolism, a review; Holman, Chandler & Cooley, 1927 - tuberculous bacilli with lead shot causing haemorrhagic infarct in dog; Hall & Ettinger, 1933 - blood-clot in dog; Hueper, 1942 - macromolecular compounds, lipids/

lipids, etc; Flory, 1945 - cholesterol crystals obtained from atheromatous human aorta in rabbit; Moses, 1946 - wool knitting yarn soaked in defibrinated dog's blood in rabbit; Harrison, 1948 - blood-clot in rabbit; Harman & Ragaz, 1950 - homogeneous liquid fat in rabbit; Wotton & Martin, Jr., 1951 - cod-liver oil saturated with Sudan IV in kitten; Gross & Brown, 1951 - English china-clay suspended in water and plasma free of fibrin in rabbit; Wartman, Jennings & Hudson, 1951 - blood-clot in rabbit; Ellis, Jr., & Grindlay & Edwards, 1952 - glass-beads in dog; Harnard, 1953 - fibrin emboli in rabbit; Pryce & Heard, 1956 - blood-clot in rabbit; Thomas, O'Neal & Lee, 1956 - blood clot in rabbit).

It has been shown also that ligation of the pulmonary artery alone does not produce pulmonary infarct. (Karsner and Ash; Ellis, Jr., et al; Schlaepfer, 1926; Liebow, et al, 1950.) Karsner and Ash; Chapman and his colleagues (1949) and Moses had observed from their experimental work that pulmonary infarct did not occur when there was interference with the arterial circulation unless it gave rise to circulatory stasis in the pulmonary segment concerned.

Records/

Records of experimental production of pulmonary infarct by chemical means can be cited from the works of Orth (see supra), Chapman and his colleagues, and Stanton and Stouffer (1957). Chapman and his colleagues produced pulmonary infarcts in 3 of 8 dogs by administering large doses of alphanaphthylthiourea (ANTU) and subsequently releasing intravascular clot into the peripheral venous circulation to work as emboli. Stanton and Stouffer produced infarction of lung by a single sub-lethal injection of hexachlorotetrafluorobutane in the marginal ear vein of rabbits and dogs.

Most of the experimental work cited above was mainly concerned with the production of pulmonary infarct and its relation to pulmonary emboli. Little attention had been paid to the study of the nature of changes of lung tissue during the healing of the infarct. Only two relevant papers of Karsner and Ash, and of Stanton and Stouffer refer to this.

In this part of my thesis infarction of lung was produced experimentally in cats and rabbits primarily to study the reparative phase of the lesion with particular attention to regeneration of lung tissue during healing of the infarct.

EXPERIMENTAL PROCEDURE

Ten healthy adult cats weighing about 5 - 6 kgm. each, and 31 rabbits weighing 1400 - 1800 gms., of both sexes were used as the experimental animals. The animals were maintained on unrestricted diet of fish and meat for cats and pelleted rabbit food and water for rabbits. Approach has been made in two different experimental methods to produce pulmonary infarct in these two groups of animals.

First Method.

(Liquid polyvinyl acetate = PVA, Cats).

PVA is an amorphous chemical substance, soluble in organic solvents. Saturated solution of PVA in acetone was used for injection. The liquid PVA thus prepared, forms a fine scum when comes in contact with water or blood. This property of the substance has been utilised for the production of pulmonary embolism by directly administering it into the pulmonary artery. Whisnant and associates (1954) and Moyes (1957) produced cerebral infarcts by injecting red vinyl acetate and liquid polyvinyl acetate, respectively, into the internal carotid artery after occluding the external carotid, occipital and pharyngeal arteries in dogs.

Experiment:/

Experiment: The cats were anaesthetised by intratracheal ether and oxygen under positive pressure anaesthesia. Thoracotomy was performed between the 6th and 7th ribs on the right side to expose the root of the right lung by retracting the ribs. One of the major branches of the pulmonary artery at the root of the right lung was selected for injection and cleaned. With a 1 cc. tuberculin syringe and a 20-gauze needle, washed with acetone, 0.30 cc. ofPVA was drawn. The needle was then introduced into the selected branch of the pulmonary artery and the PVA was injected slowly over a period ranging from 15 - 30 seconds to produce pulmonary embolism. Immediately after withdrawal of the needle the pulmonary vein of the same side was clipped with two silver 'brain-clips' to produce venous stasis in the lung. Neither leakage of PVA nor haemorrhage from the needle-hole, was a problem. Thereafter, the thoracotomy wound was closed in layers. The entire experiment was carried out under strict aseptic precautions.

The response of the animals to the operative procedure was quite satisfactory. They were observed to come round within an hour after operation and were little upset.

Second/

Second Method.

(Tetrachlorodifluoroethane = TCF, Rabbits)

Stanton and Stouffer found that hexachlorotetrafluorobutane (HCF), a halogenated hydrocarbon, produced a direct chemical injury to vascular endothelium of the lung capillary bed. Through the courtesy of Organic Department of E.I. DuPont de, Nemours & Co., Wilmington, Delaware, U.S.A., I have been able to use a related hydrocarbon, tetrachlorodifluoroethane (TCF), which also caused a direct chemical injury to the vascular endothelium of the capillary bed producing an acute endarteritis with subsequent obliterative fibrosis of the vessels in the lung. The capillary lesions, thus produced, involved the entire septal wall to produce a sudden complete necrobiosis of tissue which was the beginning of infarct.

TCF, which has similar properties as those of HCF, is not miscible with water but in common organic solvents, and when agitated in blood, it forms a course emulsion which remains dispersed for some time. It has no measurable effect on the clotting time of whole blood. It is an oily liquid at 30°C with a density of 1.200. It solidifies at a temperature below 20°C/

20°C. This property of solidification of the substance, at or below 20°C, frequently caused obstruction to the needle-hole while drawn into the syringe and during injection into the small marginal ear vein of rabbit. This drawback was, however, overcome by placing the container in a hot-waterbath at 25° - 30° and after rapidly filling the syringe with the substance in liquid state, the required quantity had to be introduced into the vein fairly quickly (0.25 ml. in 15 seconds). It was observed that TCF, at equivalent doses of HCF, caused lesions in the lung which were similar to those described by Stanton and Stouffer. (Mr. Stanton had kindly confirmed the same by personal communication).

Experiment: Rabbits were used and the TCF was administered as a single injection into the marginal ear vein of each animal. Doses, ranging from 0.01 - 0.4 ml. per kilogram body weight, were tried and the best result was obtained at the dose of 0.15 ml. per kilogram body weight and, as a rule, this dose was subsequently administered into the experimental animals.

Results.

First Method. Of 10 cats, 2 died within a few hours of operation and at autopsy their right/

right lungs were found severely congested. The remainder were killed by intraperitoneal injection of nembutal (10 cc.) at intervals of from 12 hours - 60 days. Five cats were killed at intervals of 5, 15, 30 and 60 days after operation.

Second Method. Of 31 rabbits, 7 died operatively of severe dyspnoea immediately after injection of TCF. Of the 24 that survived, 8 animals were killed within 48 hours of operation and 16 were killed at intervals of from 4 - 120 days.

Fixation and staining of tissue

Blocks of lung tissue, containing areas of infarction were fixed in suitable fixatives, namely, in 10 per cent formol-saline for haemotoxylin and eosin, elastic tissue stain, alcian blue method and for Prussian blue reaction; in cold acetone (4°C) for enzyme reaction; in modified Baker's fluid for R.N.A. and D.N.A.

After 48 hours of fixation the blocks of tissues were dehydrated in the usual manner and impregnated with and embedded in paraffin in vacuo. Paraffin embedded blocks were sectioned at 5u - 6u and the sections were stained by haemotoxylin/

haemotoxylin and eosin for routine examination. Selected sections were prepared with Hart's elastic tissue stain and for Prussian blue reaction. For histochemical study suitable sections were stained by alcian blue and P.A.S. for mucopolysaccharide; Kurnick's plasma cell stain for R.N.A. and D.N.A.; and for enzyme reactions the sections were stained after Gomori's method with little modification. Stained sections were dehydrated, cleared and mounted on Canada balsam for microscopical examination.

Macroscopical Appearance of the Lesion

With both methods, no infarcts were seen earlier than 48 hours after operation.

The macroscopical appearances of the lesion is shown in Table IV.

The rabbits killed within 18 hours of intravenous TCF, showed well demarcated areas of discrete hyperaemia, the precursors of infarcts, throughout all lobes of the lungs. Cats killed at the same intervals showed severe congestion of the right lung; probably due to the occlusion of the pulmonary vein, the congested lobe being considerably larger than normal and the embolic areas by contrast with the deeply congested/

TABLE IV

Duration of life after operation	Animal	Result
6 - 24 hrs.	Rabbit	No infarcts. Well demarcated areas of discrete hyperaemia throughout all the lobes of both lungs.
	Cat	No infarcts. Congested right lung, embolic areas were lighter in colour than the congested lung. (Fig. 218).
48 - 72 hrs.	Rabbit and Cat	True infarct developed. The above changes were more distinct, sharply defined, hyperaemic areas were elevated and apparently solid. Pleura covered with fibrin.
4 - 5 days	Rabbit and Cat	Well demarcated areas of infarcts, localised in the distal part of the lobes and pyramidal in shape. (Figs. 216, 217).
7 days	Rabbit and Cat	Cut surface of the infarct showed dry, grey areas of necrosis.
2 - 4 wks.	Rabbit and Cat	Necrosis more extensive on cut surface, brownish in colour, enclosed by fibrous capsule, became smaller in size.
3 - 4 mths.	Rabbit	Small conical areas of scar tissue were left.

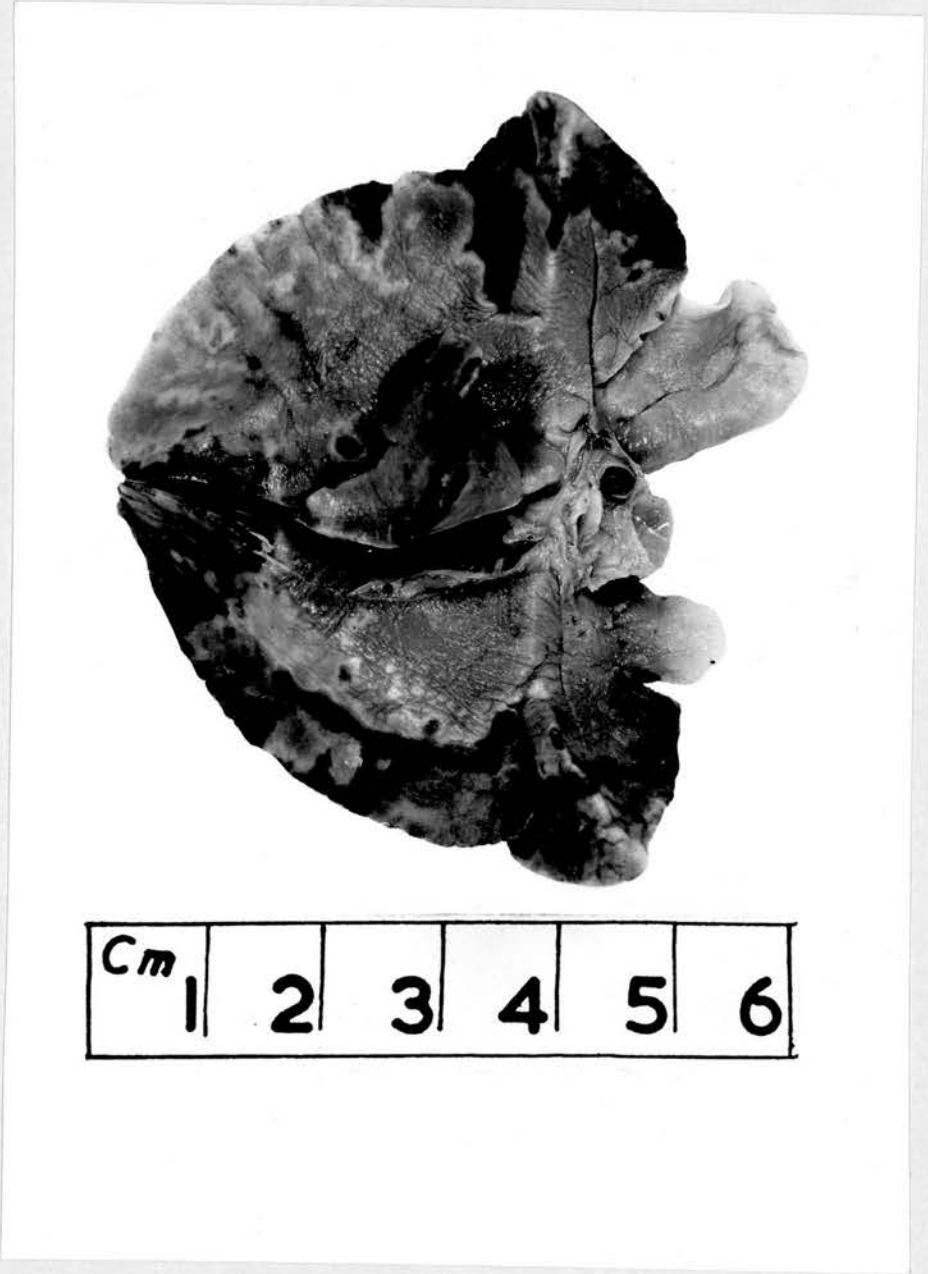

Fig.216 (Cat 37.) : 5 days old pulmonary infarct showing well demarcated areas of the lesion, localised in the distal part of the lobes involving their edges.

Fig.217 (Rabbit 13.) : 4 days after intravenous TCF, shwoing areas of infarction in the lung.

Fig.218 (Cat 33.) : 24 hours after operation. Voluminous congested right lung. Embolic areas are lighter in colour.

gested lobe appeared lighter in colour. (Fig. 218). The affected areas were, in both groups of animals, generally triangular on section of the lung, the apex being away from the surface of the lung.

Three rabbits (TCF) and two cats (PVA) were killed between 48 and 72 hours. By 48 hours the lesions in the lungs were more distinct, sharply defined and the hyperaemic areas were elevated and apparently solid. They were commonly localised in the distal parts of the lobes and involved their free edges. This peripheral location caused the lesions to be pyramidal in shape. Thepleural surface of the infarct was often covered by fibrin. When cut, the surface of these areas of infarct bulged slightly and were characteristically ischaemic lesions separated from unaffected lung tissue by a prominent border of haemorrhagic consolidation. (Figs. 219 - 222). The lesions varied in size according to the doses used. In rabbits doses of 0.15 - 0.2 ml. of TCF per kilogram body weight produced infarcts roughly 0.5 - 1.5 cm. in their greatest dimension and in cats, 0.3 cc . of PVA produced infarcts 1 - 1.5 cm. from apex to base. When 0.35 - 0.4 ml. of TCF per kilogram/

gram of body weight was injected in rabbits, the lesions were too extensive to be compatible with life and the animals, so injected, died within an hour or two. Doses less than 0.10 ml. per kilograme body weight had practically no effect on the lungs.

There was no superficial difference in infarcts 4 - 5 days duration but after one week the cut surface showed numerous dry grey areas of necrosis near the middle of the lesion. At 2 weeks the necrosis was more extensive and somewhat yellowish in colour. Subsequently the older infarcts became brownish in colour and enclosed by a fibrous capsule; the areas of infarcts correspondingly became smaller, firmer and depressed beneath pleural surface. By 4 months these lesions could be recognised only from a completely organised small conical area of scar tissue of healed infarcts. (Fig. 273).

Microscopical Appearance of the Lesion

Morphological Evolution: There were some differences in the early stage of development of the lesions in the two groups of animals specially with regards to the haemorrhages in the area of infarcts as in the first method/

method, the infarcts were produced by bland embolism with subsequent occlusion of pulmonary vein and in the second, the lesions were caused by direct chemical injury to the vascularendothelium without inducing venous congestion of the organ. Changes during the reparative phase of the infarcts and during regeneration of lung tissue were similar in both groups.

Cat (PVA): Animals, killed 6 and 12 hours after injection, showed patchy hyperaemia usually with a few red blood corpuscles in the alveoli but sometimes with definite haemorrhagic consolidation. The alveolar capillaries were stuffed with erythrocytes. (Fig. 226). By 24 hours the larger blood vessels of the lung were stuffed with red blood corpuscles: some of the injected material could be seen in these vessels and in oedematous fluid of the alveoli. (Fig. 225). The congestion extended to subpleural region though there was little alveolar haemorrhage by 24 hours. By 48 - 72 hours, definite microscopic evidence of infarction had developed. In the defined area of infarct, haemorrhage had become very extensive with rupture of some alveolar walls and desquamation of alveolar epithelium. The red blood corpuscles/

les were packed tightly into the alveoli (Fig. 238) and many of them were poorly defined and feebly stained. (Fig. 229). In the haemorrhagic area, the alveolar walls were indistinct and their nuclei had disappeared. The larger septa showed only slight degenerative changes at this stage (Fig. 233), and the large vessels were seen to contain fibrinous thrombi. Blood and fibrinous clot also filled the bronchi of the infarct (Fig. 238), but the bronchial epithelium stained normally. The capillaries in the overlying pleura were congested.

In the cat killed on the fifth day, the pulmonary infarct was well-defined and larger than those in the earlier cats, and there were alveolar phagocytes with pigment granules in their cytoplasm. Red consolidation was advanced and while the remains of degenerated nuclei might occasionally be found in the necrotic zone, the alveolar walls had usually lost all traces of nuclear structure, being represented merely by narrow, hyaline, acidophilic bands. Polymorphonuclear leukocytic infiltration was found in moderate degree in the border between infarcted and non-infarcted lung, also beneath the pleura and about the large vessels within the infarcted/

infarcted area.

In 72 - 96 hours, fibroblastic reaction was seen in the boundary zone, and around the large blood vessels of the infarct. (Fig. 234). Serosal cells had multiplied in the overlying pleura and had become elongated to resemble fibroblasts. (Figs. 229, 230 and 231 - rabbit).

Mitotic activity was found in the fibroblasts (Fig. 233), and particularly in the serosal cells of the pleura (Figs. 229, 230, 231) where marked proliferation was taking place with the formation of capillaries.

By the end of the week, the infarcted area showed the established changes of coagulative necrosis of the alveolar walls and bronchi with proliferation of fibroblasts (Fig. 235) and serosal cells. The red blood corpuscles of the alveolar spaces no longer stained with eosin and many macrophages with pigment granules were to be found. (Fig. 232).

Much of the elastic tissue of the alveolar walls was relatively disorientated and the contour of the walls was often lost in the necrotic zone. (Figs. 247 - 15 days).

Cats and rabbits: From the fifth day through the first week, replacement of the necrotic pulmonary/

monary tissue by young connective tissue, was well underway. This took place by the invasion of granulation tissue of new capillaries and fibroblast cells from areas of connective tissue proliferation in the pleura, from the surviving haemorrhagic zone at the periphery of the infarct, and from the larger septa within the necrotic area. The process of organisation progressed rapidly and in 2 - 3 weeks, except a small area near the centre, the whole necrotic zone was replaced by organising connective tissue. (Figs. 245, 246). This was completed by 4 - 5 weeks. (Fig. 253, 254). Decolourisation of the areas of haemorrhage was well marked by second week in the central part of the infarct though granular pigmentation was present at the margin of the decolourised area.

Rabbit: The onset and the necrotic phenomena of the infarcted areas in the rabbit's lung in the early stages were similar to those described above. Vascular changes were most marked and characteristic in the periphery of the chemically-produced lesions. In 6 - 12 hours swelling of the endothelium of the blood vessels in the peripheral part of the haemorrhagic band was first to occur. (Fig. 224). The capillaries/

ries were filled with masses of barely distinguishable conglutinated redcorpuscles. By 24 hours vascular endothelial changes were more intensified and involved damage to the endothelium of veins draining the areas of infarction as well as of the arteries. The vessels were lined by swollen, elevated endothelial cells and their walls infiltrated with polymorphonuclear leukocytes. The internal elastica appeared intact and the muscular walls were not necrotic outside the areas of infarction. (Fig. 227). By the fourth day and onwards the surrounding pulmonary vessels of the zone of necrosis were infiltrated with leukocytes through the vascular wall and into the perivascular space where the reaction was at its peak. Endothelial damage extended well beyond the haemorrhagic zone and fibrinous thrombi were seen on the damaged endothelium but were not occlusive in the early phase. Occlusive thrombi were found in the damaged vessels in the latter part of the first week. (Fig. 236). In course of time many of the thrombosed vessels became canalised (Figs. 245, 260) and others were narrowed by endarteritis obliterans. (Fig. 266). The/

The vessels which were involved in the scar tissue showed perivascular fibrosis. (Fig. 265).

Unlike the infarcts of the lung in cats (with venous congestion) the infarcts of rabbit's lung were not packed with red blood corpuscles as there was no venous congestion present. The infarct was virtually an area of ischaemic necrosis resembling those of an organ nourished by end arteries. Haemorrhage was unusual though the alveolar capillaries were stuffed with red corpuscles in the early stage. The small arterioles were also involved in similar way at the periphery of the zone of hyperaemia. The alveolar spaces remained clear. (Fig. 224).

By 48 - 72 hours the hyperaemic areas merged to form semi-transluscent areas of ischaemic necrosis, demarcated from grossly normal lung by a band of haemorrhagic tissue. (Figs. 219 - 222). In the areas of infarction the capillaries were filled with conglutinated red corpuscles. The alveolar walls were infiltrated with polymorphs and mononuclear leukocytes and the nuclei of the alveolar cells were pyknotic. Oedematous fluid filled the alveolar spaces (Figs. 231, 240), in contrast with that seen in cats/

cats, where the alveoli were packed with red blood corpuscles. (Fig. 229, 238). The whole picture was like that of coagulative necrosis (Fig. 240) with poor nuclear staining character in the alveolar walls.

In 72 - 96 hours organisation started from the periphery to the centre (Fig. 228) and it progressed rapidly to completely replace the necrotic zone within 3 - 4 weeks after the onset of the lesion.

Regeneration of Lung Tissue.

Evidence of regeneration of lung tissue was seen before fibrosis had occurred. The process was similar to that seen in the healing lung-wounds in cats. First in importance was the hyperplasia and proliferation of bronchial epithelium directed towards the zone of necrosis from the surviving boundary zone. The epithelium of large bronchi, situated in the necrotic area of the infarct, became hyperplastic and proliferated (Fig. 237, 239) to form bronchial buds which, in their turn, invaded the organised part of the infarct.

Mitotic activity was quite frequent in the proliferating epithelium (Figs. 237, 239, 241,

242) which continued in these hyperplastic cells, from the early part of repair (48 - 96 hours) as long as 4 months after the original injury. Mitoses were also present in abundance in the epithelial cells of bronchial buds and in cubical cells lining the new alveoli. (Figs. 244, 267, 270, 279).

The bronchial buds, formed from the epithelium of parent bronchi, at first grew as a solid mass of cubical cells outside the membrana propria of the bronchus and became canalised by the air pressure from the parent stem. (Figs. 267, 248, 243, 249, 264). They then gave rise to further bronchial buds by themselves (Figs. 270, 275, 279) and gradually grew deeper and deeper into the organised area of the infarct where new alveoli were formed from these buds to aerate the lung tissue. (Figs. 276, 271, 264, 250). The bronchial buds were mostly lined by cubical cells (Figs. 248, 274) though sometimes by columnar cells (Figs. 270, 256) which might be even ciliated in the more mature regenerated bronchi. (Fig. 275). Regenerated alveoli from bronchial buds were lined by cubical or flattened, elongated cells. (Figs. 250, 262, 271, 276). These lining cells of bronchial buds and /

and new alveoli stained deeply. The new alveolar spaces in the regenerated area often contained cellular debris with actively phagocytic macrophages (Figs 258, 259).

The process of regeneration was progressive and in 1 - 3 months time scar tissues of healed infarcts were seen almost completely pervaded by bronchial buds and irregular new alveoli. (Figs. 253, 255, 263 - 265). Besides this major regenerative process two other subsidiary developments occurred as were seen in the healing of experimental wounds. One was the development of irregular slits by splitting of collagen and the other, the re-expansion of collapsed alveoli. Irregular slits were seen to develop in dense scar tissue lined by cubical or flattened, elongated and spindle-shaped cells. Some of these new air spaces appeared to communicate with blood vessels and contained blood cells. (Fig. 278). Others clearly connected with bronchial buds or new alveoli, and had cubical epithelium in their wall. In the air spaces partially compressed by marginal sclerosis, the hyperplastic foci of epithelial cells were formed of compressed respiratory channels lined with ciliated columnar (Fig. 269) or hyperchromatic/

chromatic cuboidal epithelium; the two forms were frequently continuous.

Alveolar foetalisation was noted also in pulmonary parenchyma separated from the scar by normal lung tissue. Atypical stratified squamous metaplasia was not a very uncommon sight in areas of active proliferation of bronchial epithelium or of the epithelium of bronchial buds. (Figs. 251, 257, 268).

The elastic tissue in the necrotic zone was decreased, and there was some fragmentation of connective tissue (Fig. 247); new bronchial buds lacked elastica and in some instances, the appearance of the condensation of bronchi in the periphery of older infarct suggested new formation of elastic tissue (Fig. 279).

Regeneration of lung tissue thus developed in various ways during the reparative phase of pulmonary infarcts in cats and rabbits and in 3 - 4 months the lesions were seen almost completely healed by regeneration of the parenchymal tissue leaving behind only a small conical area (Fig. 273) or a thin band of scar tissue as the remnant of the infarct. On closer examination, Fig. 273 reveals the dense scar tissue/

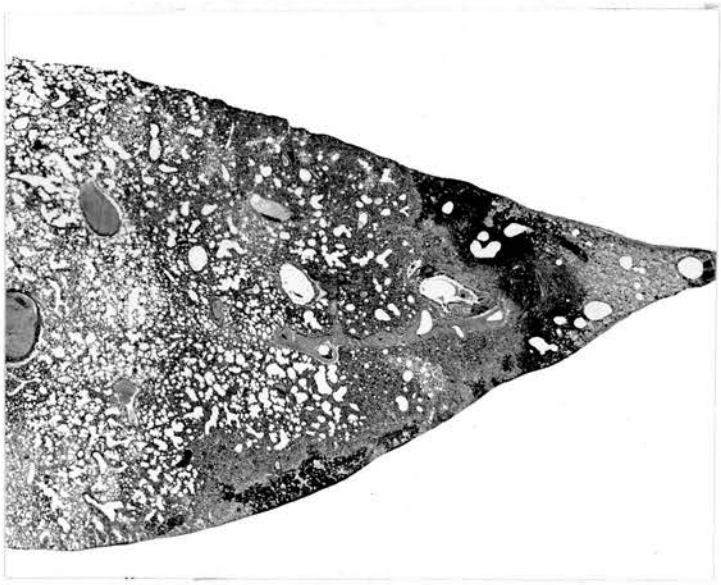

Fig.219 (Rabbit 13.) : 4 days after intravenous TCF. Section of an infarct showing from right to left : zone of coagulative necrosis, dark haemorrhagic boundary zone and the apparently healthy lung. Two blood vessels(left) are seen packed with red blood corpuscles. x 6.

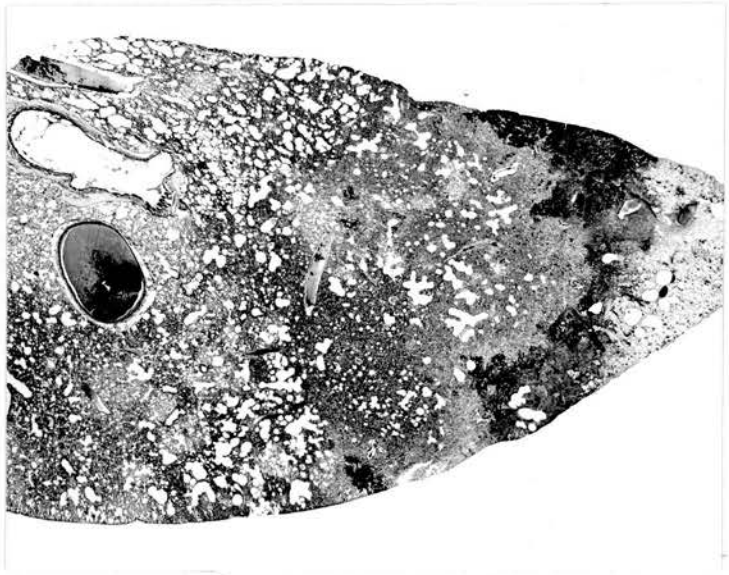

Fig.220 (Rabbit 15.) : 5 days after intravenous TCF. Right to left : zone of coagulative necrosis, haemorrhagic boundary zone and grossly normal lung. One large vessel is seen packed with blood. x 6.

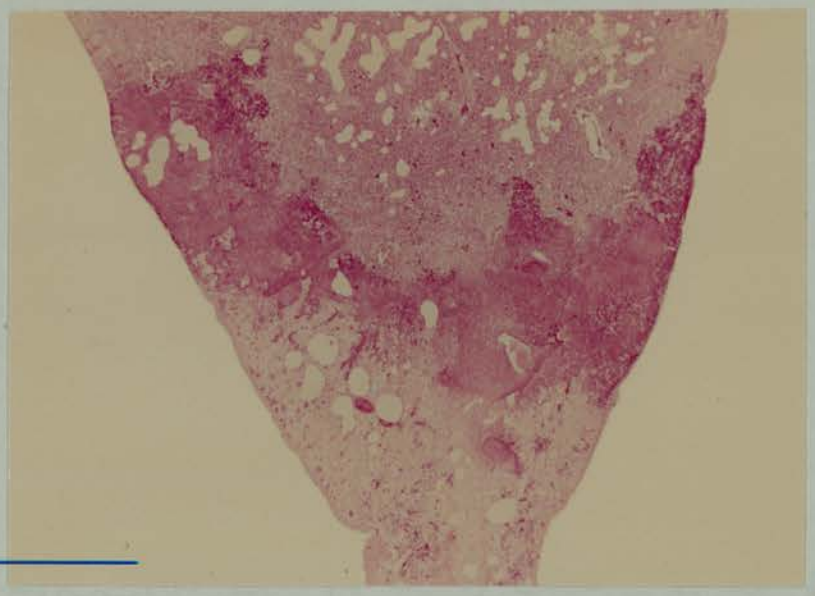

Fig.221 (Rabbit 15.) : Same case as above showing the different zones of the infarct. x 10.

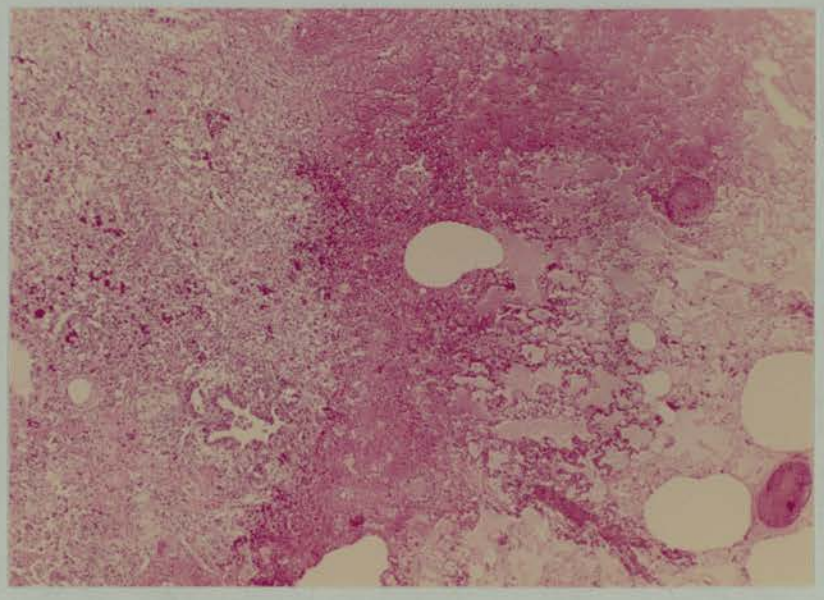

Fig.222 (Rabbit 15.) : High power of Fig.221, to show the alveoli in the necrotic zone(right) filled with oedematous fluid. Left : haemorrhagic boundary zone and grossly normal lung. x 35.

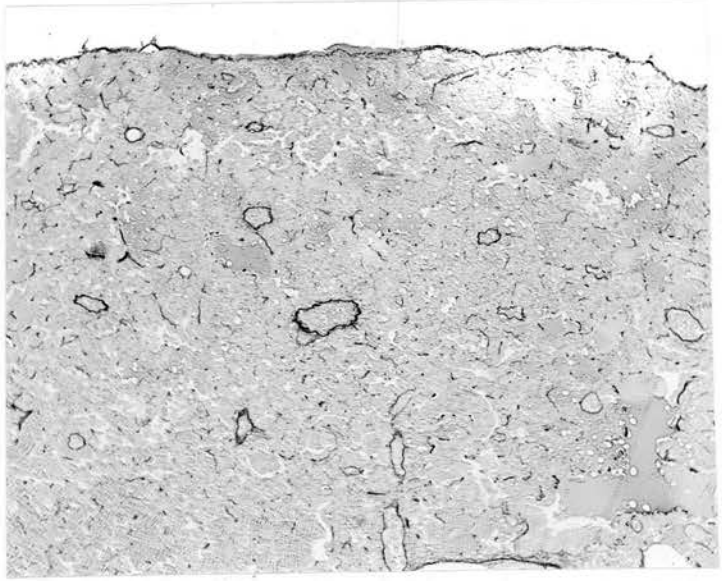

Fig.223 (Rabbit 15.) : 5 days old pulmonary infarct. The elastic tissue of the alveolar walls is relatively disoriented in the necrotic zone. x 70.

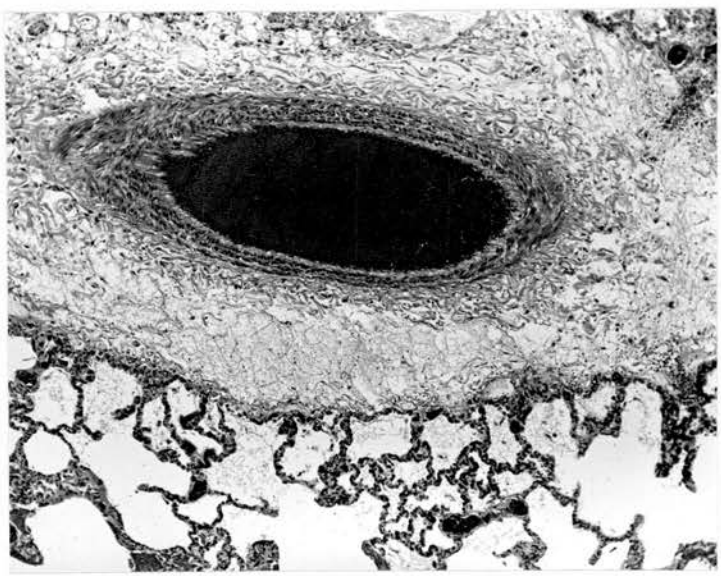

Fig.224 (Rabbit 6.) : 12 hours after intravenous TCF. A large artery is seen packed with blood. The endothelium of the vessel is swollen. Capillary hyperaemia in the alveolar wall. Clear alveolar spaces. x 65.

Fig.225 (Cat 32.) : 18 hours after operation. The lumen of the vessel is stuffed with blood corpuscles. Some of the injected material can be seen in the vessel and in oedematous fluid of the alveoli. x 55.

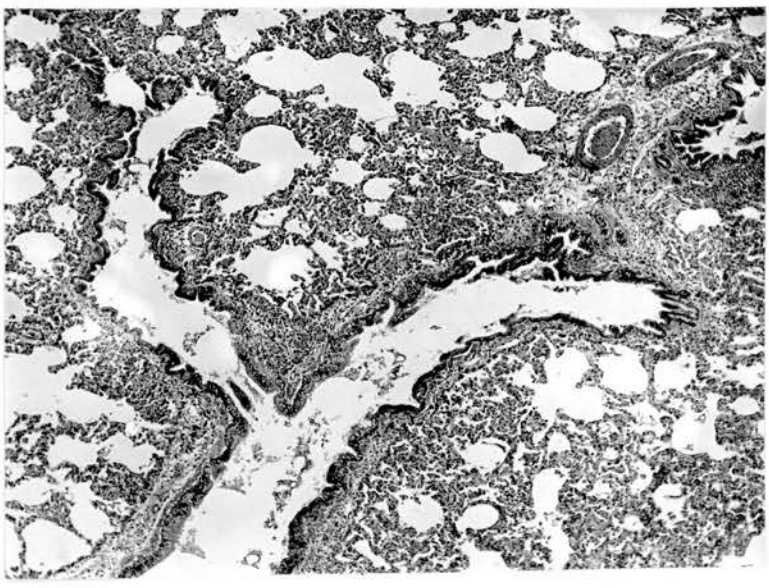

Fig.226 (Cat 33.) : 24 hours after operation. The alveolar capillaries are stuffed with erythrocytes. Small vessels are seen packed with blood. x 45.

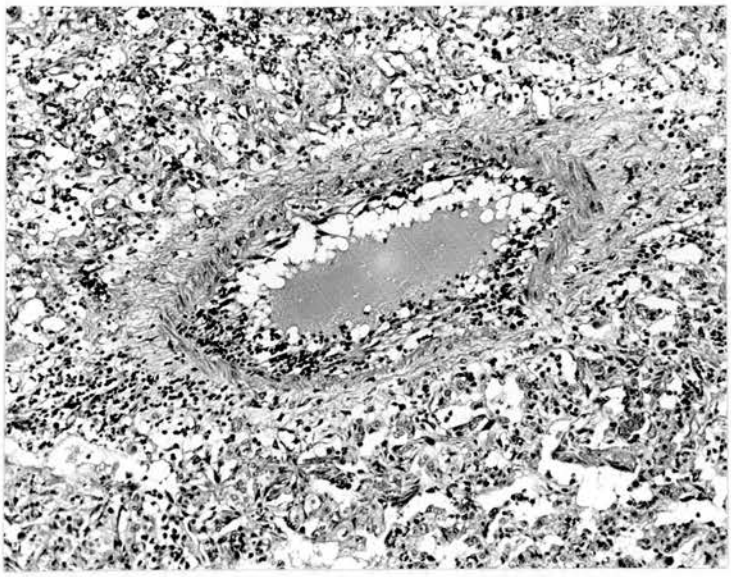

Fig.227 (Rabbit 12.) : 72 hours after intravenous TCF. The endothelium of the blood vessel is damaged. The wall of the vessel is infiltrated with polymorphs. The internal elastic lamina and the muscular wall are intact. x 120.

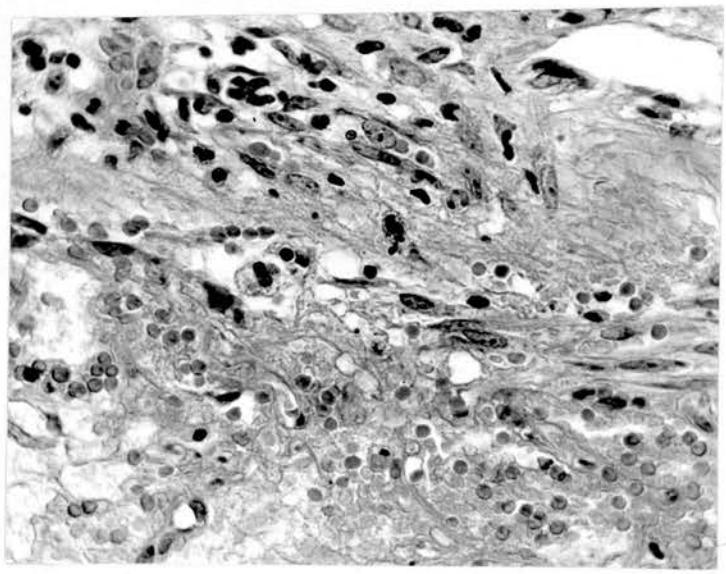

Fig.228 (Rabbit 13.) : 4 days after intravenous TCF. Young fibroblastic reaction at the periphery of the necrotic zone. x 500.

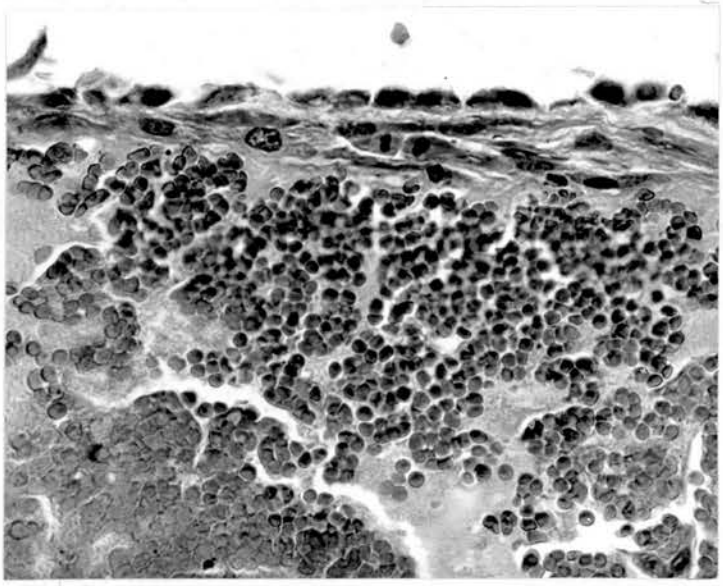

Fig.229 (Cat 37.) : 5 days old haemorrhagic infarct. The red blood corpuscles are tightly packed into the alveoli, some of them are poorly defined and feebly stained (bottom left). Serosal-cell reaction with mitotic activity in the pleura. The cells are becoming elongated to resemble fibroblasts. x 500.

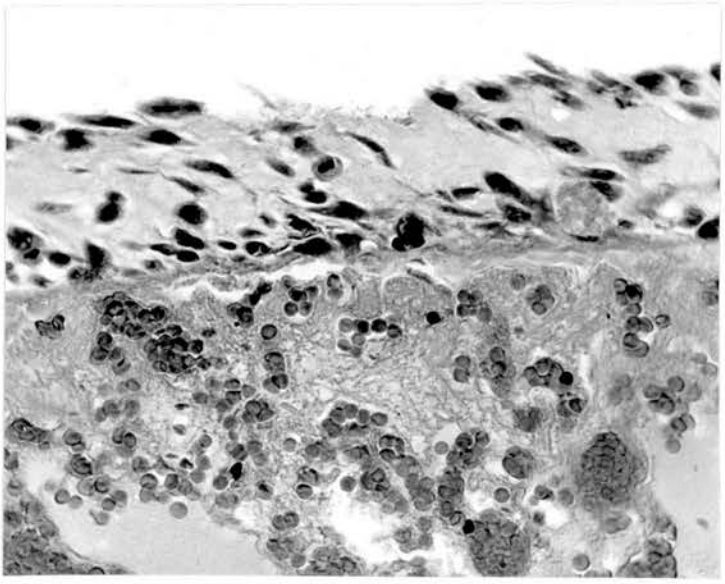

Fig.230 (Rabbit 14.) : 5 days after intravenous TCF. Serosal-cell reaction with mitotic activity. The cells have assumed elongated, spindle-shaped form and resemble fibroblasts. x 500.

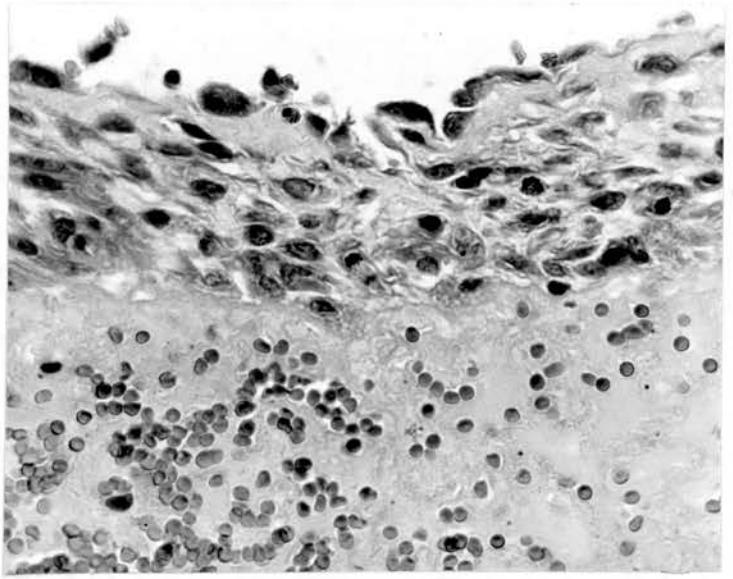

Fig.231 (Rabbit 14.) : Same case as above showing vigorous serosal-cell reaction in the pleura. The alveolar spaces are filled with oedematous fluid and few red blood corpuscles. (cf.229 - cat). x 500.

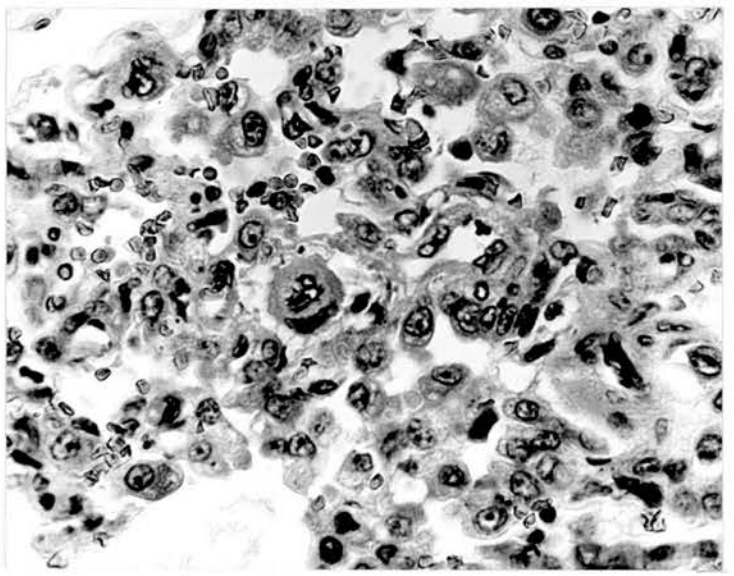

Fig.232 (Cat 37.) : 5 days old pulmonary infarct showing actively phagocytic macrophages in the necrotic zone. x 550.

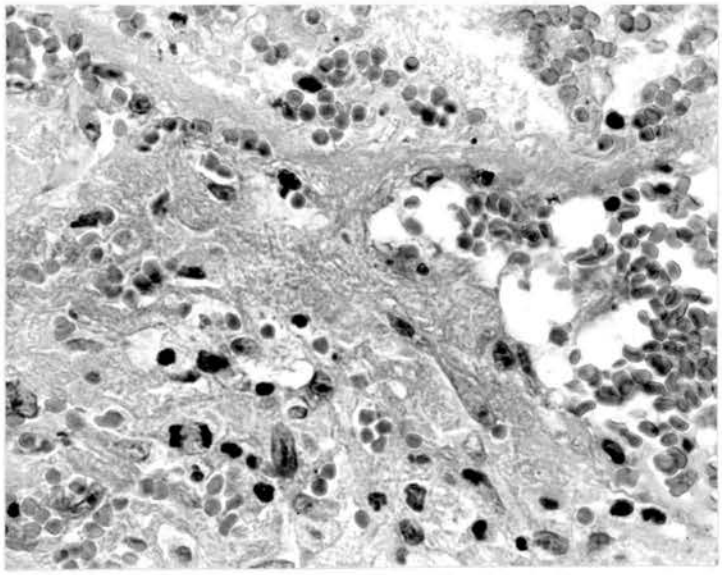

Fig.233 (Cat 37.) : 5 days old pulmonary infarct. Mitotic activity is seen in the area of early fibroblastic reaction. Slight degenerative changes present in the large septum of the lung in the necrotic zone. x 525.

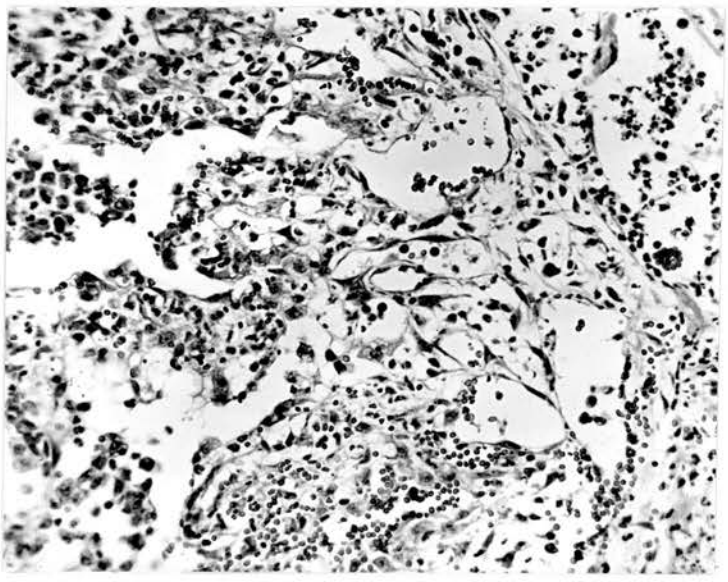

Fig.234 (Cat 37.) : 5 days old pulmonary infarct. Early fibroblastic reaction in the boundary zone. x 250.

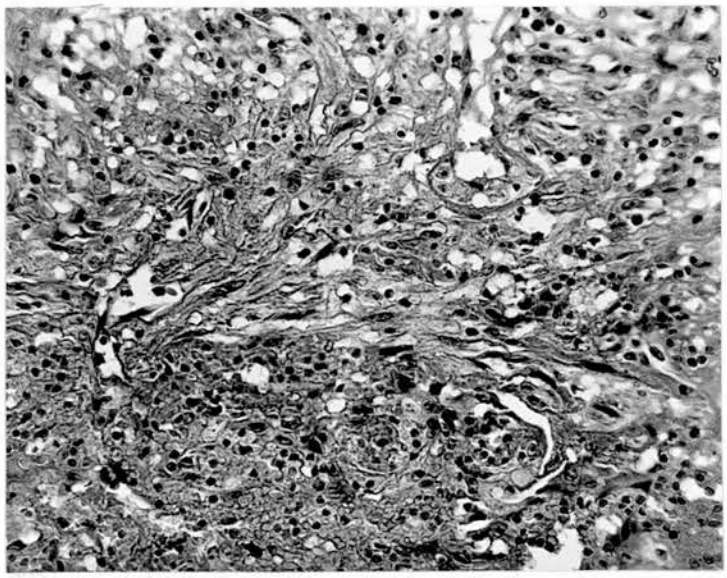

Fig.235 (Cat 37.) : 5 days old pulmonary infarct. Zone of necrosis with proliferation of fibroblasts from larger septa. x 250.

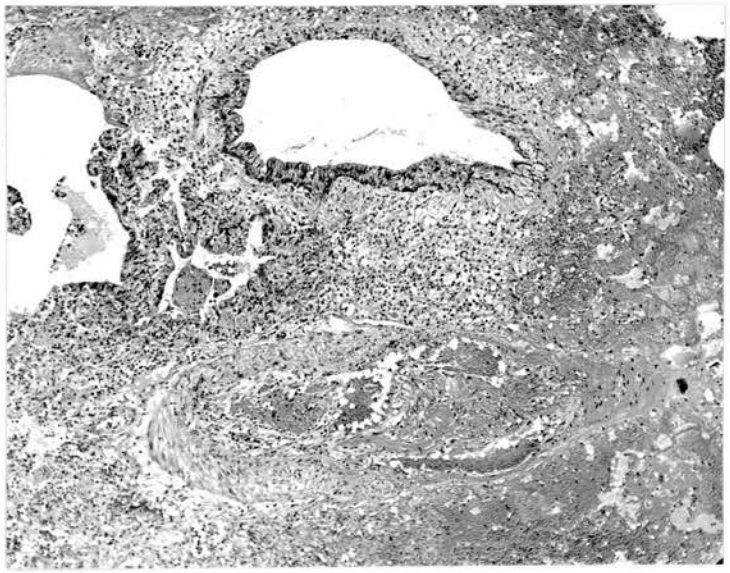

Fig.236 (Rabbit 15.) : 5 days after intravenous TCF. Occlusive thrombus in a damaged vessel in the necrotic zone of the infarct. The muscular wall of the vessel is damaged. x 65.

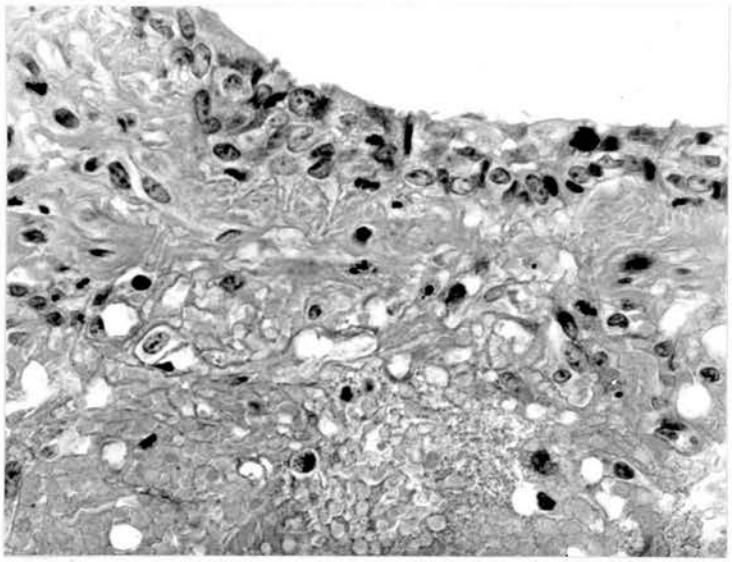

Fig.237 (Cat 37.) : 5 days old pulmonary infarct. Hyperplastic and proliferative epithelium of a large bronchus in the necrotic zone with mitotic activity in the proliferating cells. x 500.

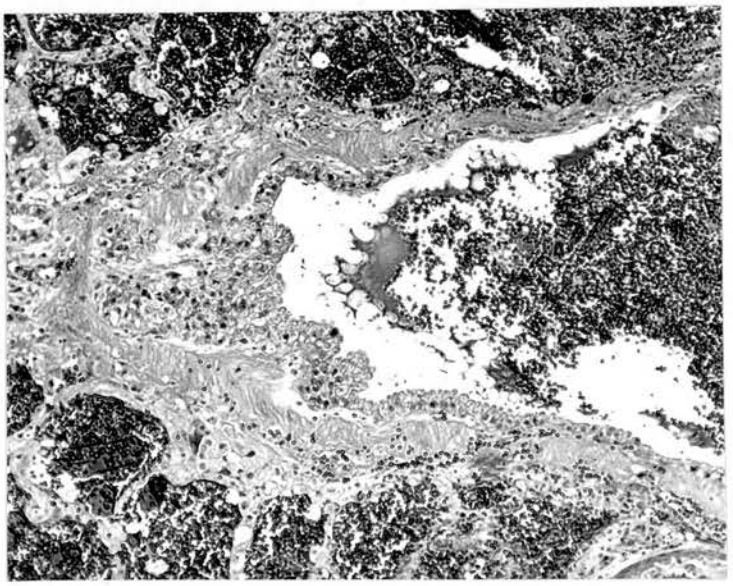

Fig.238 (Cat 37.) : 5 days old pulmonary infarct. The alveolar spaces are packed tightly with erythrocytes. The lumen of a large bronchus is filled with blood and fibrin-clot with normal lining epithelium. x 150.

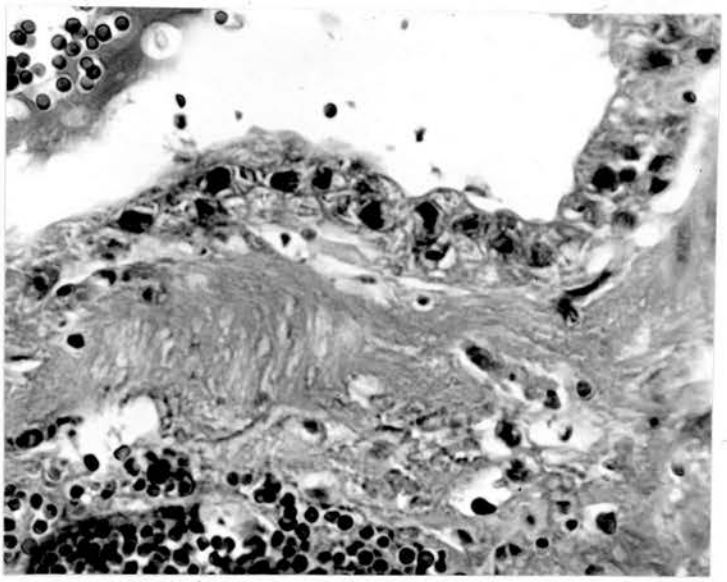

Fig.239 (Cat 37.) : High power view of Fig.238, to show the proliferating bronchial epithelium with mitotic activity. x 600.

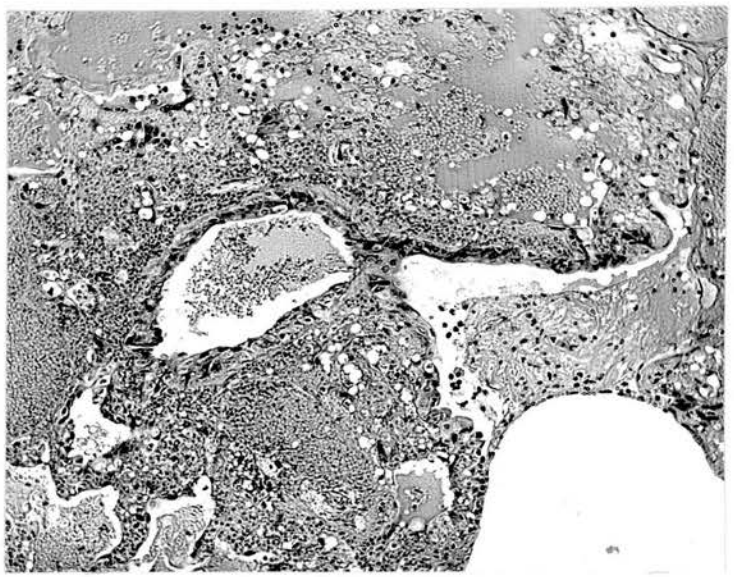

Fig.240 (Rabbit 16.) : 7 days after intravenous TCF. The bronchial lumen and alveolar spaces are filled with oedematous fluid with poor nuclear staining character in the alveolar walls. x 130.

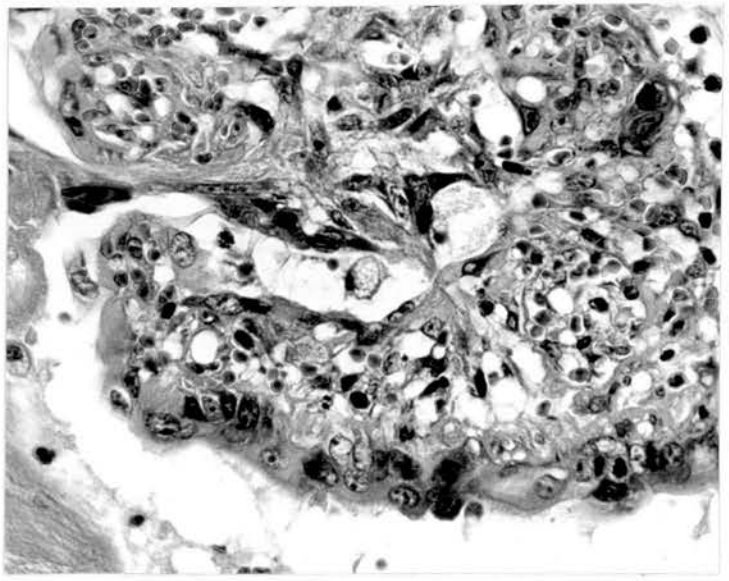

Fig.241 (Rabbit 16.) : High power of Fig.240, to show the proliferative activity of the bronchial epithelium, with mitotic activity, in necrotic zone. x 500.

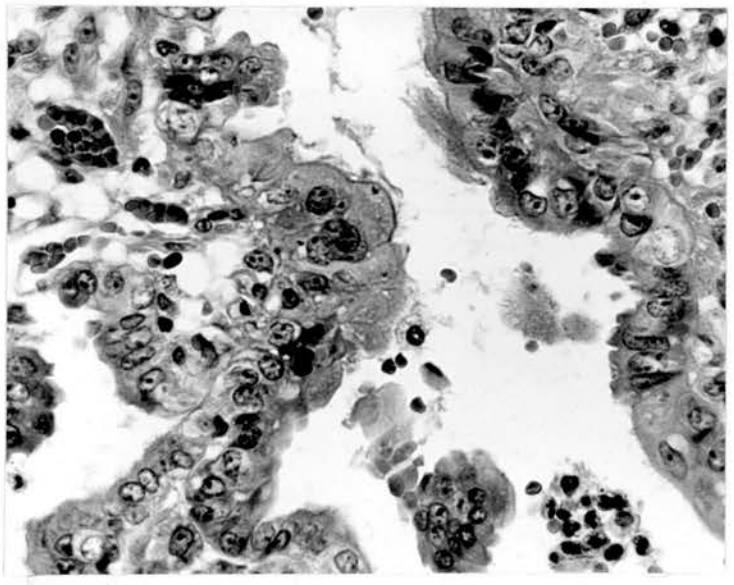

Fig.242 (Rabbit 17.) : 7 days after intravenous TCF. Proliferative activity of bronchial epithelium, with mitotic activity, in the zone of necrosis. The lummina of the bronchus is filled with cellular debris. x 500.

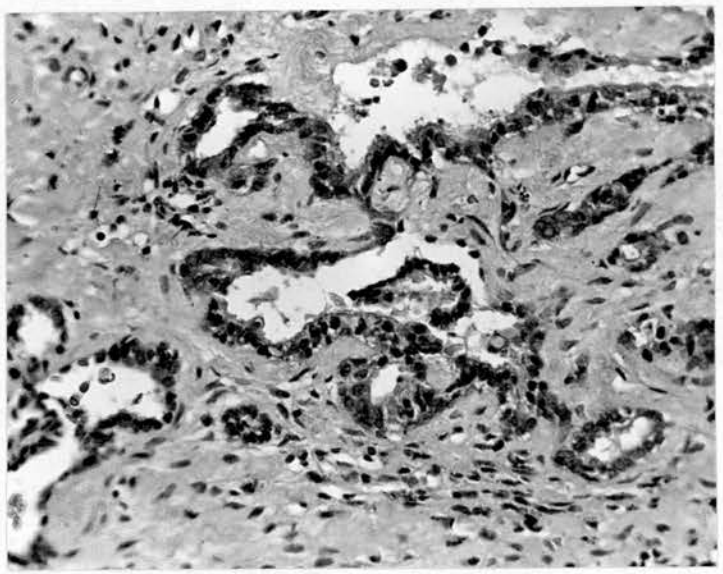

Fig.243 (Rabbit 17.) : 7 days old pulmonary infarct showing bronchial budding in the organised area of the lesion. x 275.

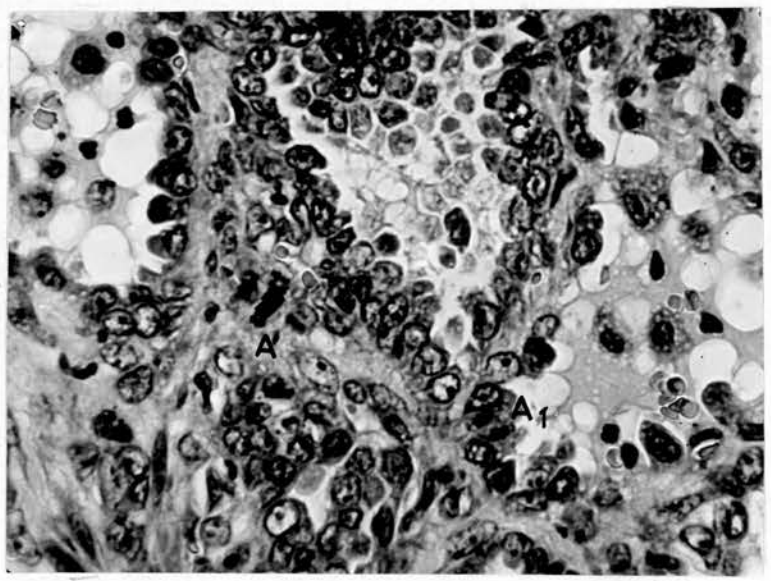

Fig.244 (Rabbit 16.) : 7 days old pulmonary infarct. Hyperplastic plump cuboidal cells lining the alveoli with mitoses (A, A_1). x 600.

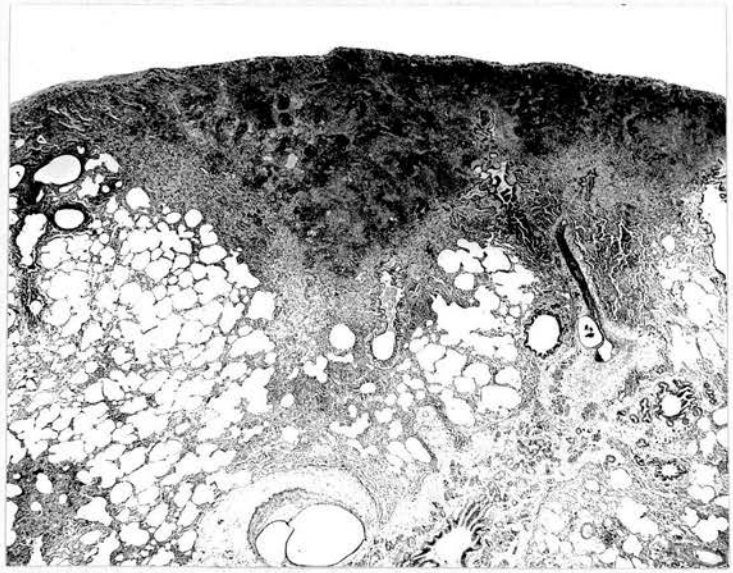

Fig.245 (Cat 38.) : 15 days old pulmonary infarct, showing organisation of the necrotic zone from the periphery of the lesion. Bronchial buds are seen growing into the organised area. A re-canalised thrombosed blood vessel is seen in the periphery of the infarct. x 13.

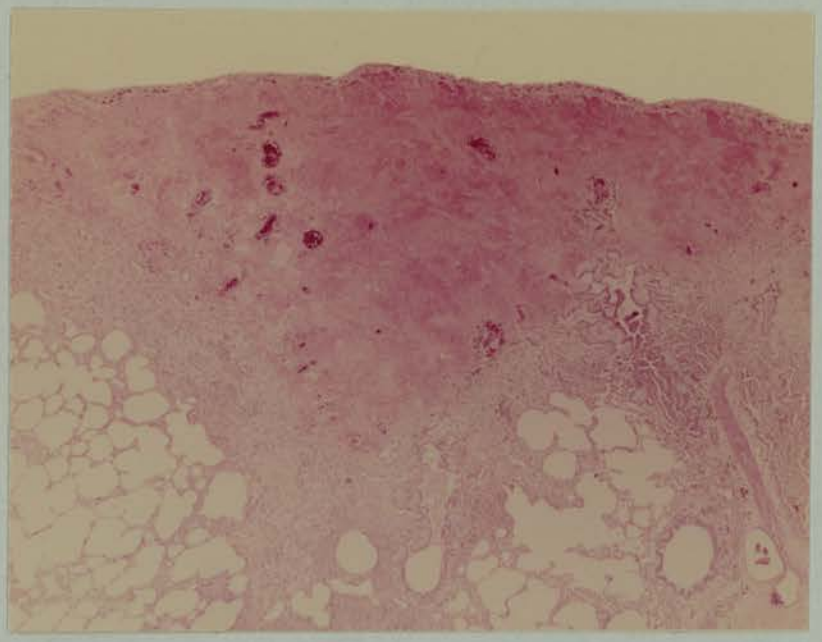

Fig.246 (Cat 38.) : Same case as above to show the gradual replacement of the necrotic zone by organising tissue with bronchial budding in the periphery of the lesion. The deeper zone is still haemorrhagic. x 22.

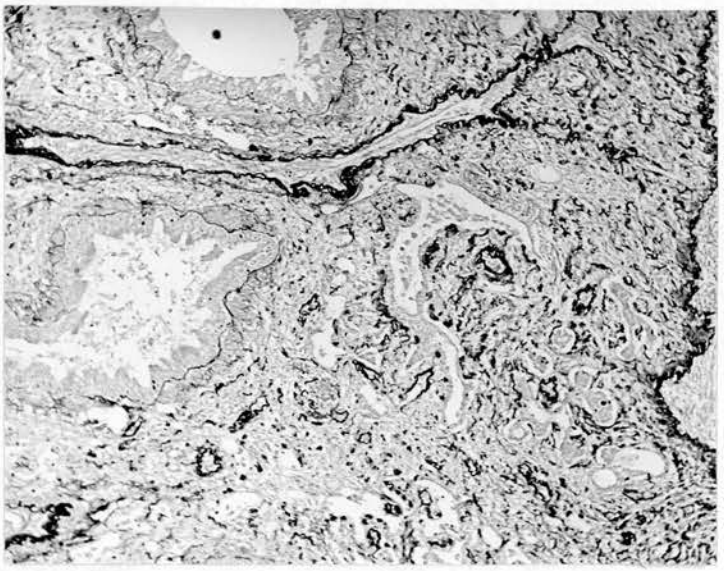

Fig.247 (Cat 38.) : 15 days old pulmonary infarct showing partial disorganisation of the elastic tissue of the alveolar wall and some fragmentation of connective tissue. x 70.

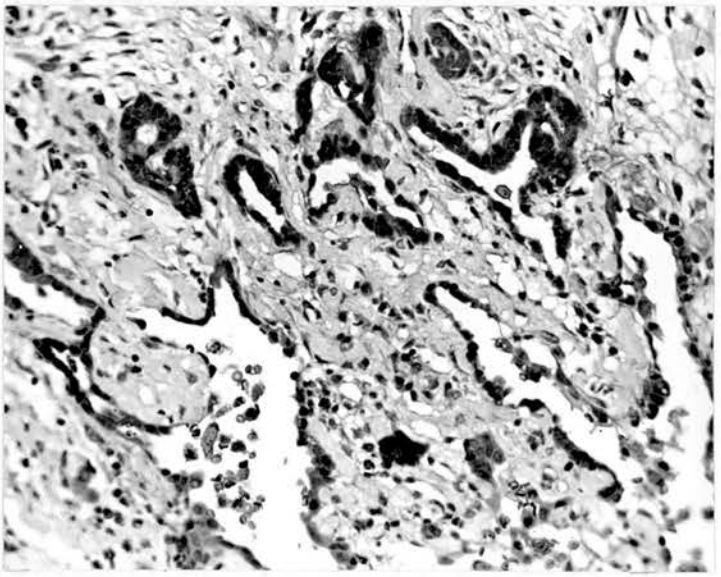

Fig.248 (Cat 38.) : 15 days old pulmonary infarct showing bronchial budding which are mostly lined by cubical cells. x 275.

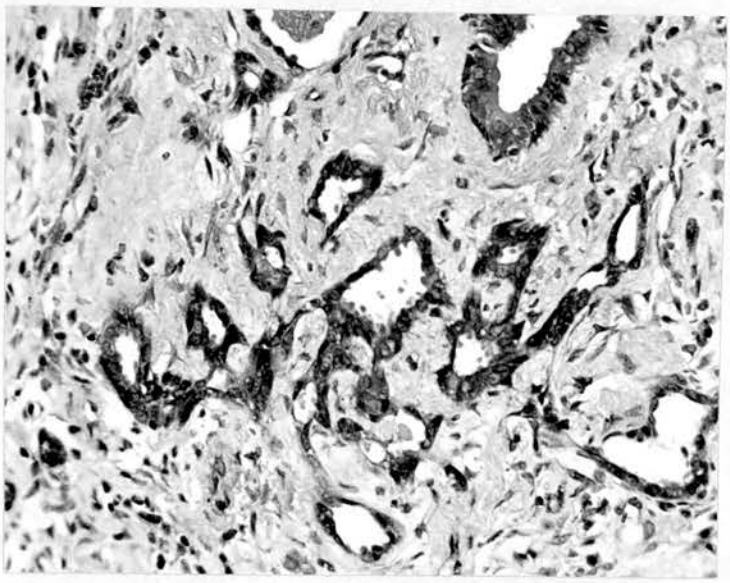

Fig.249 (Rabbit 18.) : 15 days old organised infarct showing a regenerated bronchus (top right) and bronchial buds. x 270.

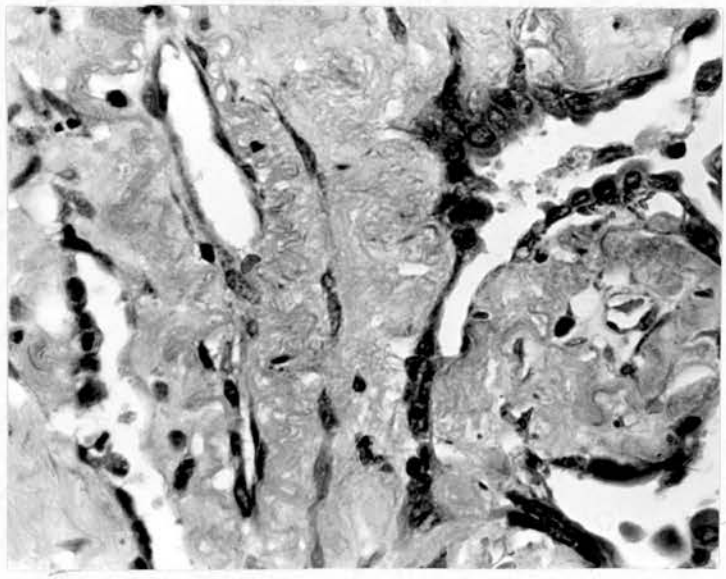

Fig.250 (Rabbit 19.) : 15 days old pulmonary infarct. A regenerated bronchus is seen giving rise to bronchial buds and alveolar formation. Some of the newly-formed alveoli are lined by flattened, elongated cells. x 525.

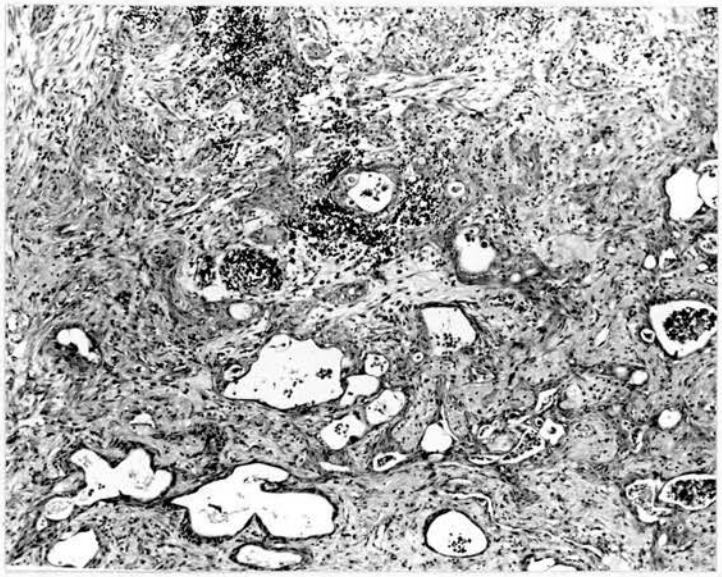

Fig.251 (Cat 38.) : 15 days old pulmonary infarct showing an area of active proliferation of epithelial cells with squamous type of metaplasia. x 75.

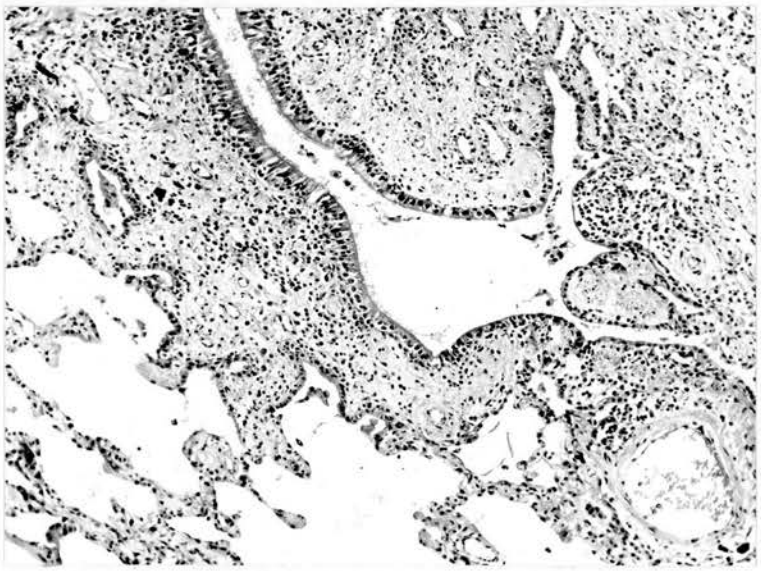

Fig.252 (Cat 38.) : 15 days old pulmonary infarct, showing a longitudinal section of a bronchus at the periphery of the organised area. The canalised bronchial buds are lined by cubical cells. x 110.

Fig.253 (Cat 39.) : 30 days old pulmonary infarct. The infarcted area is replaced by fibrous tissue scar which is pervaded by numerous bronchial buds and new alveoli. x 13.

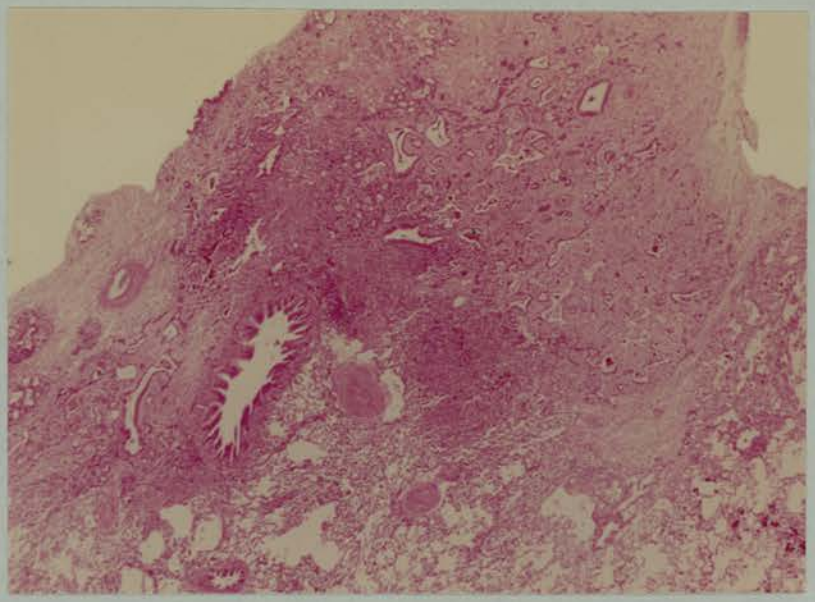

Fig.254 (Cat 39.) : Same case as that of Fig.253, to show bronchial budding and alveolar formation throughout the whole area of the infarct which is completely transformed into scar tissue. x 22.

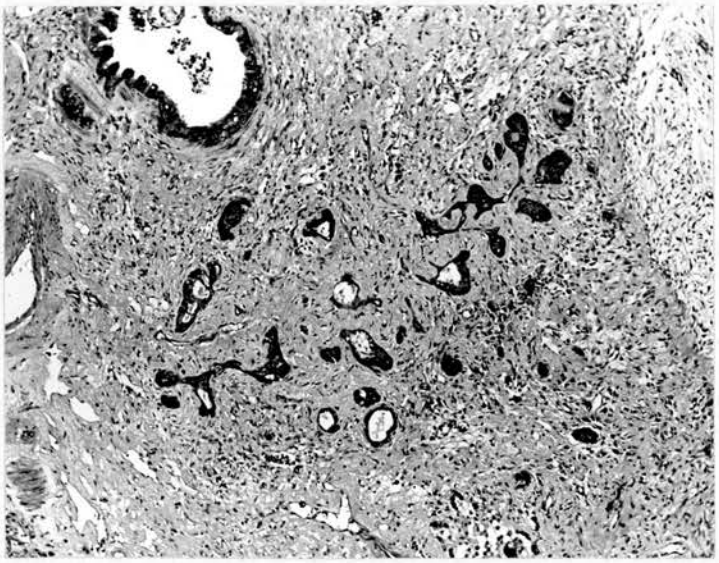

Fig.255 (Cat 39.) : High power of Fig.253, to show bronchial buds in the scar tissue. A newly-formed bronchus, lined by low columnar cells, is seen in the field (top left). x 175.

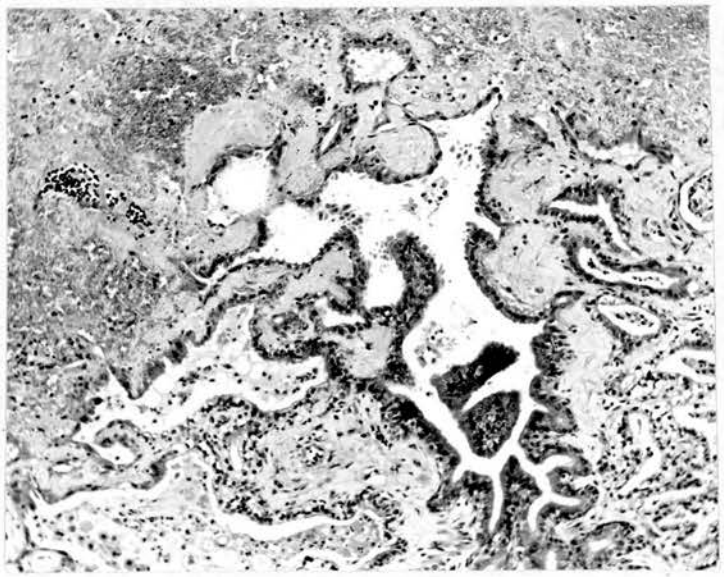

Fig.256 (Cat 39.) : Bronchial budding and alveolar formation in the organised part of the infarct. Part of the epithelial lining is low columnar in type. x 110.

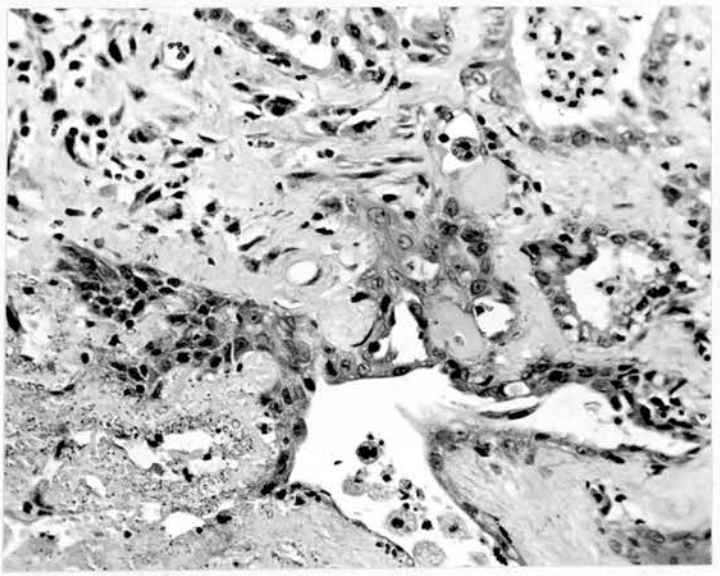

Fig.257 (Cat 39.) : High power of Fig.259, to show the stratified squamous type of epithelial metaplasia in the organised area of the infarct. x 275.

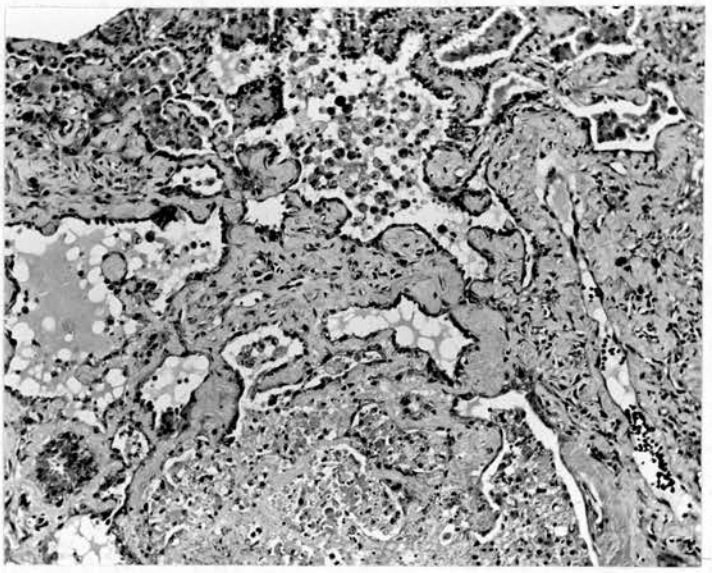

Fig.258 (Cat 39.) : 30 days old pulmonary infarct. The newly-formed alveoli are filled with necrotic debris with phagocytic macrophage cells. x 110.

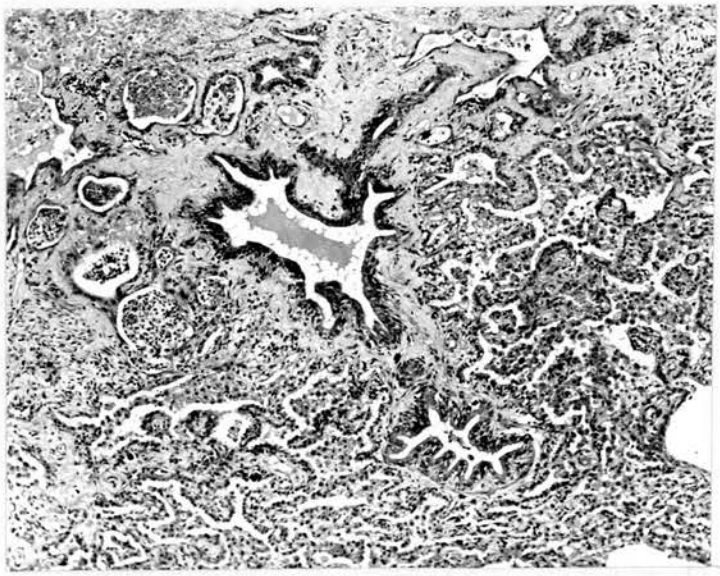

Fig.259 (Cat 39.) : 30 days old pulmonary infarct showing an area of regenerated lung tissue with a formed bronchus which is giving rise to new bronchial buds. The alveolar spaces contain cellular debris with actively phagocytic macrophages.
x 75.

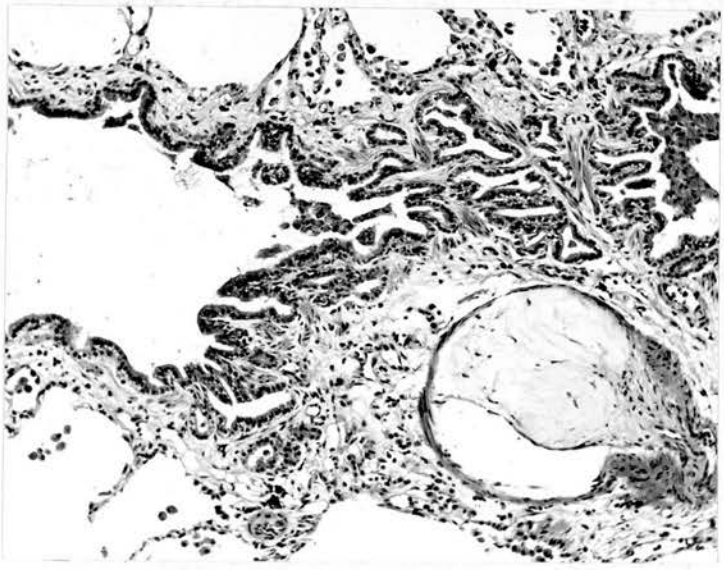

Fig.260 (Rabbit 22.) : 30 days old pulmonary infarct. A re-canalised thrombosed vessel is seen at the periphery of the lesion with bronchial buds in the surrounding area.
x 110.

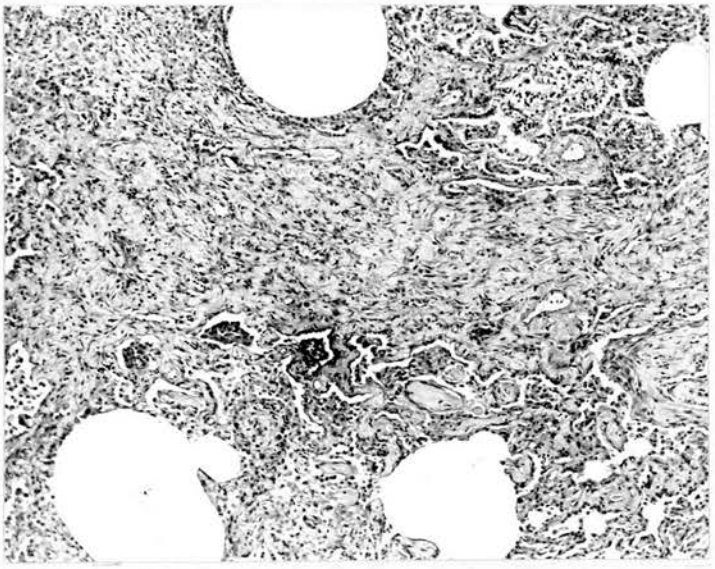

Fig.261 (Rabbit 22.) : 30 days old pulmonary infarct, showing an area of regenerating lung tissue. x 75.

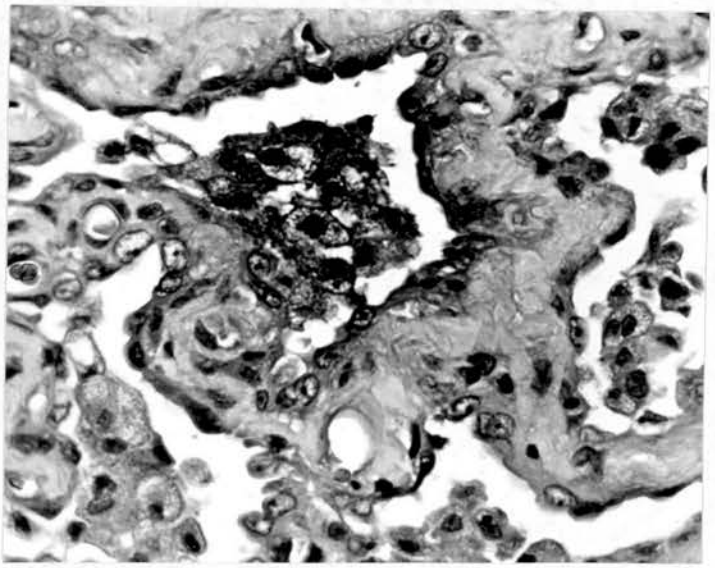

Fig.262 (Rabbit 22.) : High power of Fig.261, to show the alveolar cell-lining - some are cubical and others flattened and elongated x 525.

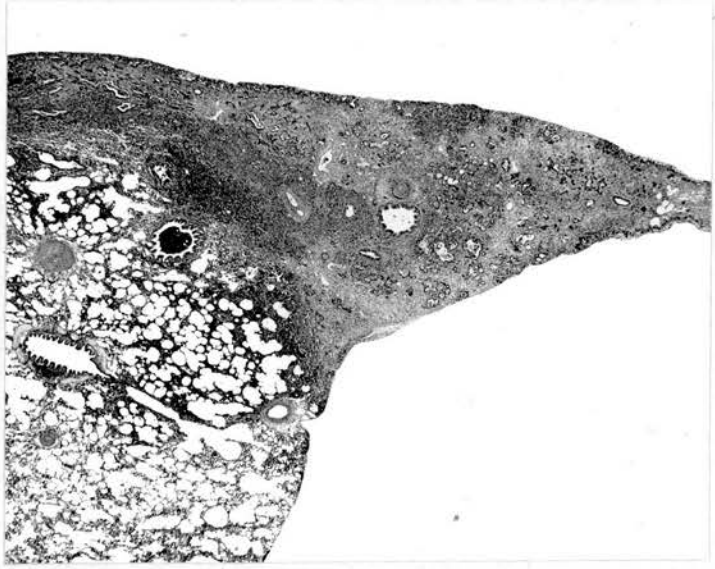

Fig.263 (Rabbit 24.) : 60 days old pulmonary infarct. The infarcted area is replaced by scar tissue which is pervaded by bronchial buds and new alveoli all over. x 13.

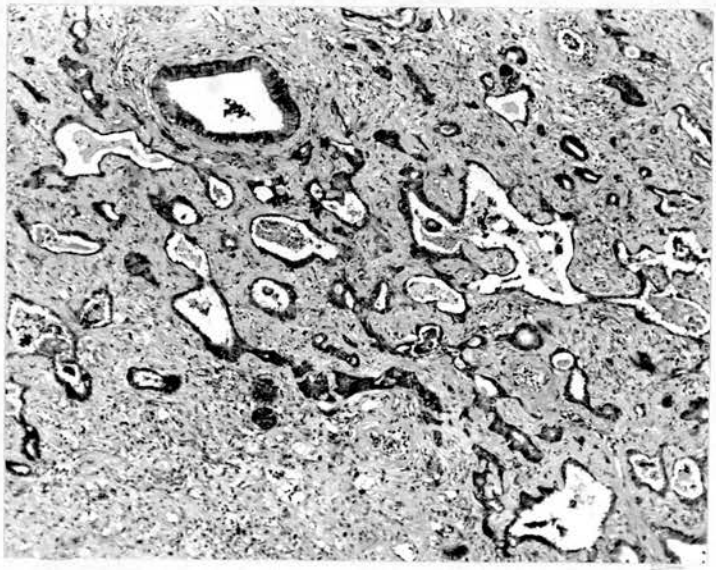

Fig.264 (Rabbit 24.) : High power of Fig.263, showing numerous bronchial buds and new alveoli in the dense fibrous tissue of the lesion. x 75.

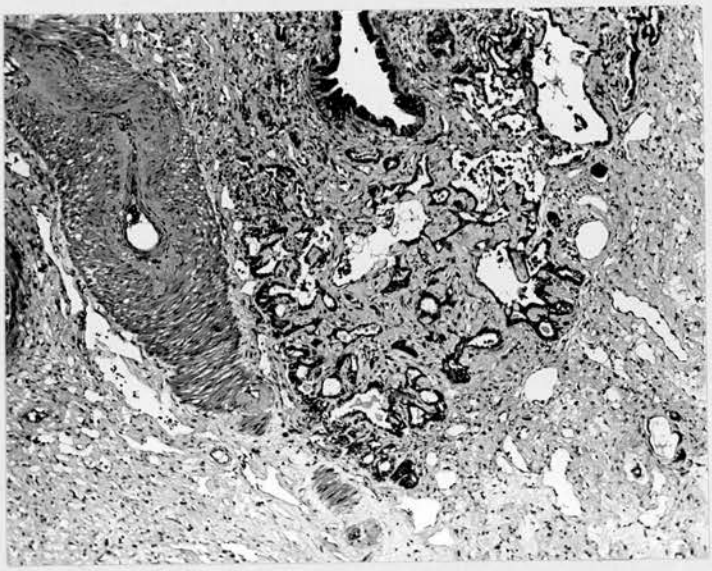

Fig.265 (Rabbit 24.) : High power of Fig.263, to show bronchial buds and alveoli in the scar tissue. A blood vessel is seen with endarteritis obliterans and perivascular fibrosis. x 75.

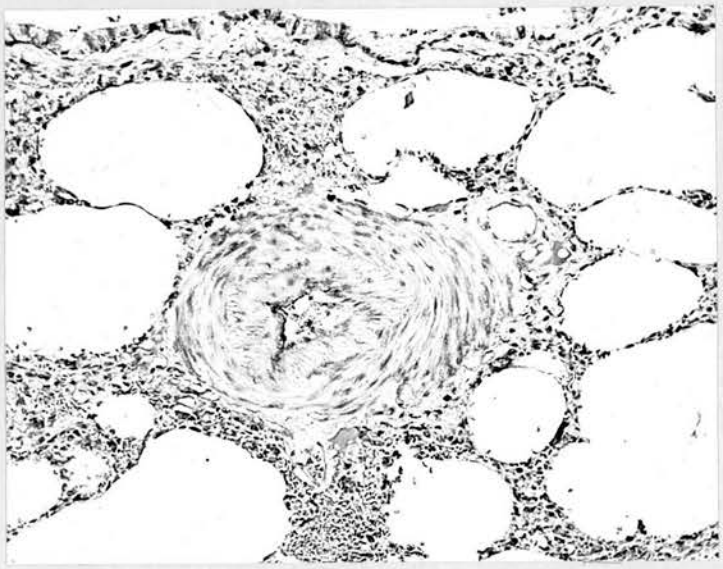

Fig.266 (Rabbit 24.) : 60 days after intravenous TCF. One of the damaged blood vessels is narrowed by endarteritis obliterans. x 140.

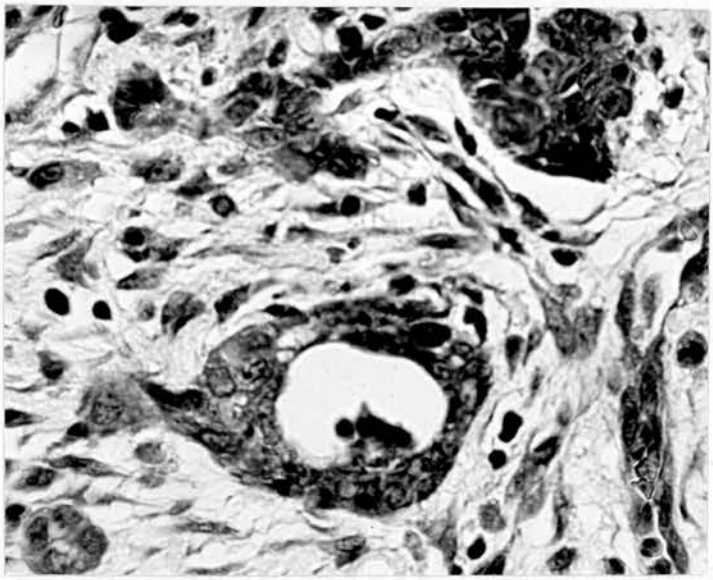

Fig.267 (Rabbit 24.) : 60 days old pulmonary infarct. A canalised bronchial bud, lined by cubical cells with mitosis, is giving rise to further epithelial bud in the organised part of the lesion. x 550.

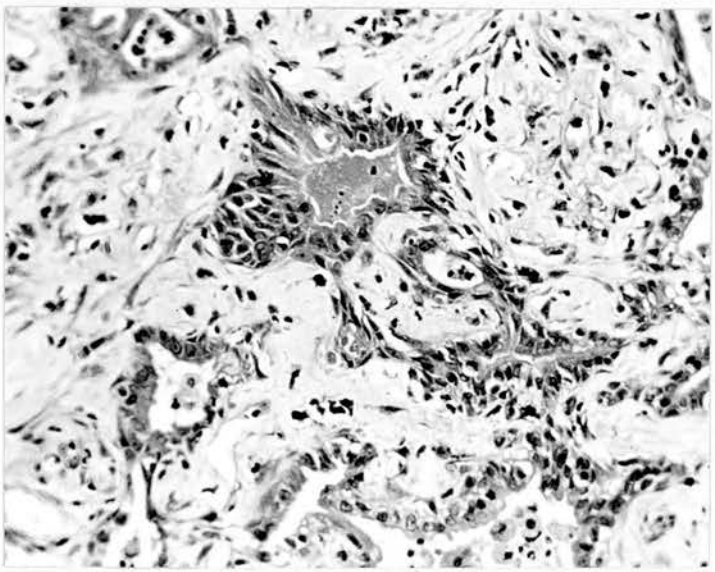

Fig.268 (Cat 40.) : 60 days old pulmonary infarct. Atypical stratified squamous metaplasia in an area of active proliferation of bronchial epithelium. x 250.

Fig.269 (Cat 40.) : 60 days old pulmonary infarct showing re-expansion of compressed alveoli. Some are lined by ciliated low columnar cells. x 110.

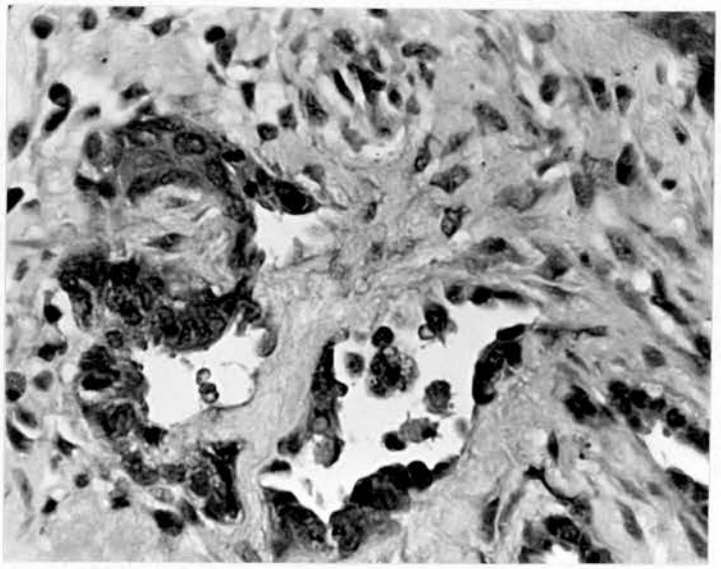

Fig.270 (Rabbit 25.) : 90 days old pulmonary infarct showing formation of bronchial buds and new alveoli. Some lining cells are columnar in type. Mitotic activity is seen in the new bud. x 525.

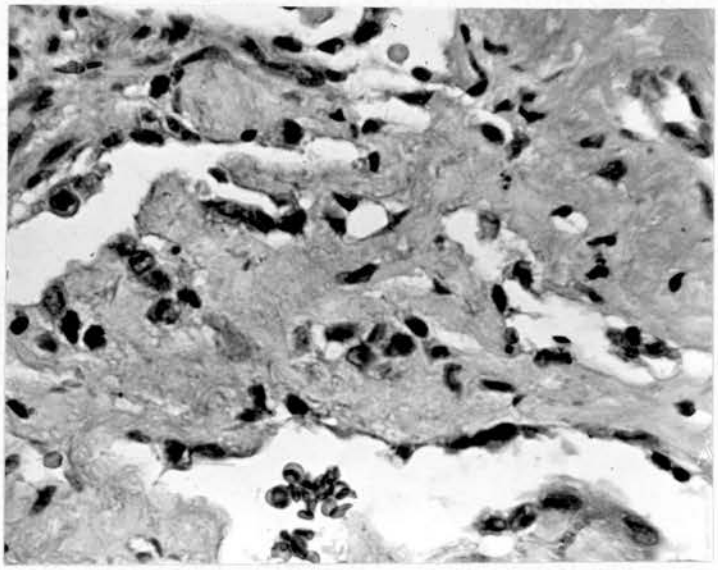

Fig.271 (Rabbit 25.) : 90 days old pulmonary infarct showing regenerated alveoli lined by flattened, elongated cells. x 525.

Fig.272 (Rabbit 25.) : 90 days old pulmonary infarct. The appearance of the condensation of bronchi in the periphery of older infarct suggests new formation of elastic tissue. x 150.

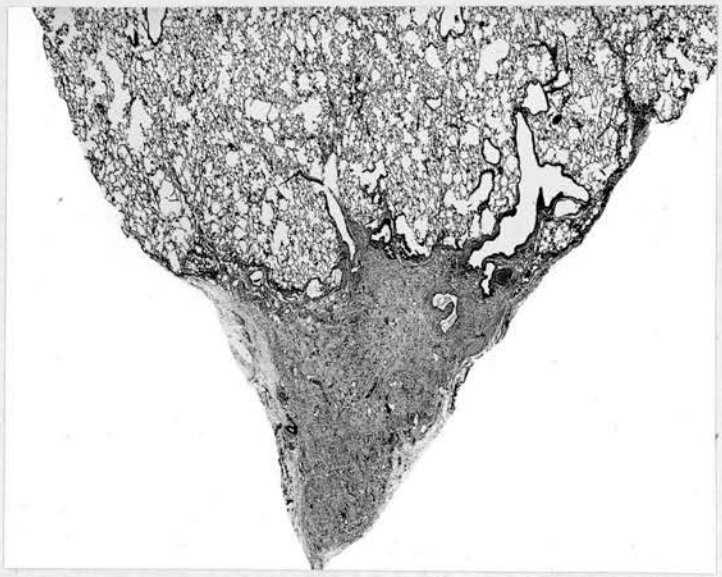

Fig.273 (Rabbit 26.) : 104 days old pulmonary infarct. The conical area of scar tissue of the healed infarct is invaded by bronchial buds from the peripheral region. x 13.

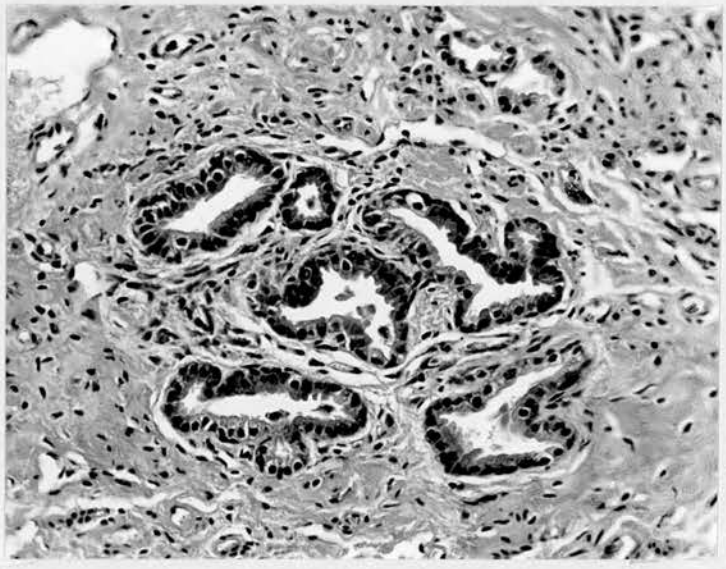

Fig.274 (Rabbit 26.) : High power view of bronchial buds lined by cubical cells, invading the scar tissue of the infarct, illustrated in Fig.273.

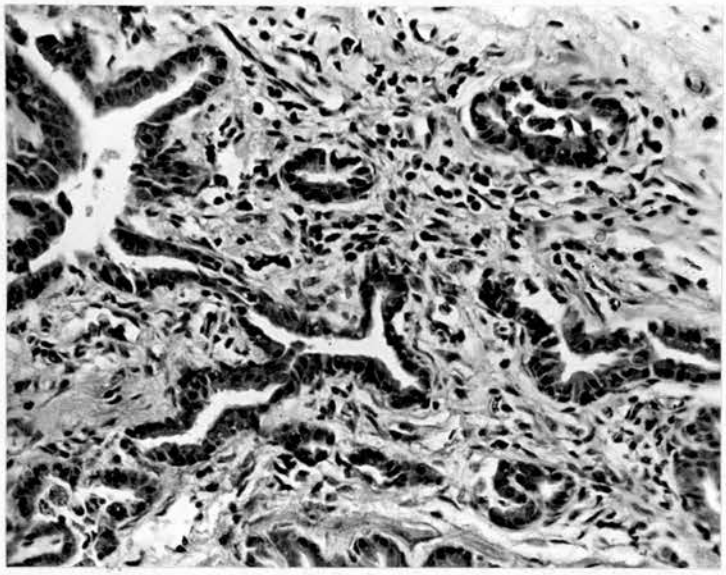

Fig.275 (Rabbit 28.) : 120 days old pulmonary infarct showing bronchial buddings in the scar tissue. Some lining cells of these new buds are ciliated low columnar in type (top left). x 275.

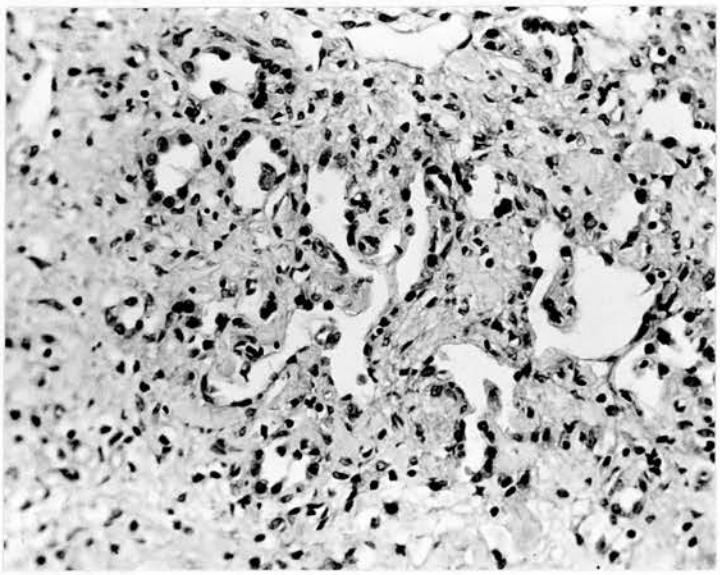

Fig.276 (Rabbit 27.) : 120 days old pulmonary infarct showing new alveolar formation lined by cubical cells. x 275.

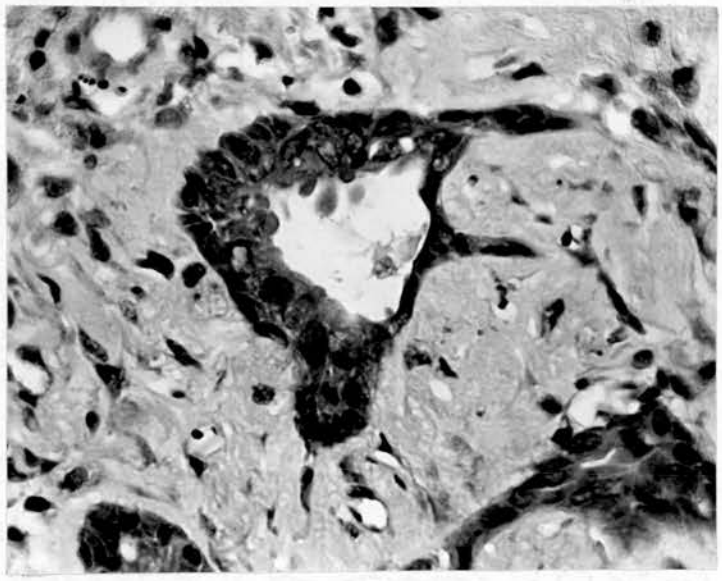

Fig.277 (Rabbit 27.) : 120 days old pulmonary infarct showing bronchial buds with mitotic activity in the lining epithelium. x 525.

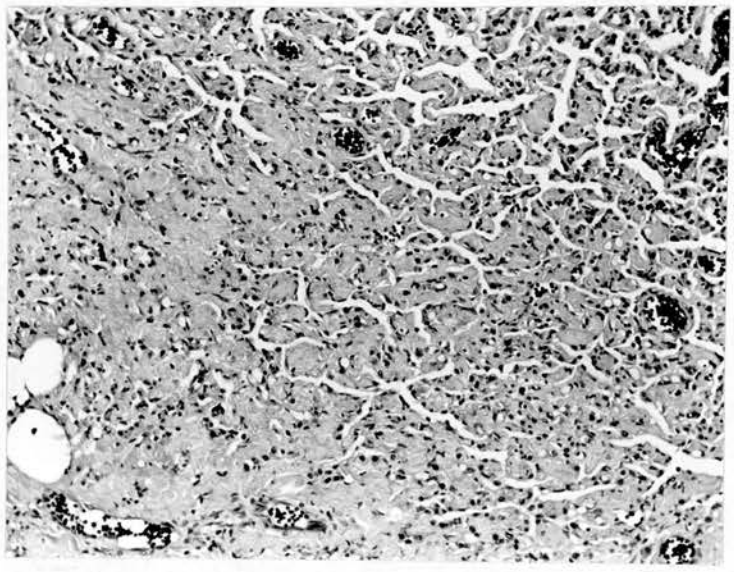

Fig.278 (Rabbit 27.) : 120 days old pulmonary infarct. The slit-like air channels are formed by splitting of collagen in the scar tissue of the infarct. They are lined by cubical or flattened, elongated cells. Some of these new air channels communicate with blood vessels and contain blood. x 130.

tissue in conical shape, there are no bronchial buds or new alveoli (cf.Figs. 253 & 263) in the central part of the scar but at the inner margin, bronchial buds are seen eroding the fibrous tissue (Fig. 274) which would ultimately be replaced by regenerated lung, though if not wholly at least partially.

Histochemical Reactions of the Regenerating Tissue

As compared with normal controls (Figs. 172, 173, 174 & 175) the epithelium of the bronchi outside the necrotic area of the infarct gave positive staining reactions for enzymes and R.N.A. and D.N.A., similar to those seen in healing wounds of lung. These reactions were only faintly positive in the epithelial cells of bronchi in the infarcted zone where necrotic process was fairly advanced.

The regenerating bronchial buds and alveoli in the organised part of the infarct show very mild reactions for alkaline phosphatase (Figs. 279 - 281) and non-specific esterase (Figs. 284, 285) with a fairly stronger intensity for acid phosphatase. (Fig. 282).

The swollen and proliferated alveolar lining/

lining cells, in some parts of the organised area, were observed to give intense staining reactions for esterase (Fig. 286) and acid phosphatase, (Fig. 283).

Similarly, R.N.A. and D.N.A. reactions were mildly positive in the lining cells of bronchial buds and new alveoli (Fig. 287) in the regenerating area of the lung.

Alcian blue reaction for mucopolysaccahride remained constantly negative in the epithelial cells of bronchial buds and new alveoli but in areas of fibroblastic reaction this reaction was always positive (Fig. 288).

DISCUSSION.

The above experimental work on the production of pulmonary infarcts by means of chemical agent in rabbits, confirms the findings of Stanton and Stouffer that true anaemic infarcts without superimposed haemorrhage can be produced chemically in the lung by directly injurying the lung capillary bed and septal walls. In support of this conclusion were the facts that the infarcts were seen to develop prior to formation of any occlusive thrombi in the large vessel and before any formidable damage to the muscular/

muscular walls of these vessels became visible. The direct action of the chemical agent (TCF) to the endothelium of the capillaries caused a condition of total cessation of pulmonary circulation to the part concerned, which ultimately led to the production of a true ischaemic infarct in that area of the lung.

This ischaemic infarct in rabbits did not differ materially from the haemorrhagic ones, produced embolically in the congested cats' lung, where the lesions were first hyperaemic, then haemorrhagic and finally became pale from coagulative necrosis - the haemorrhagic phase being absent in the infarcts produced chemically in rabbits.

Karsner and Ash concluded from the results of their experimental work that although organisation was rapid in an uncomplicated pulmonary infarct, there was no evidence of any attempt whatever at regeneration of either the alveoli or bronchi in experimentally produced infarcts of dogs' lung. Stanton and Stouffer in discussing the phenomenon of persistent hyperplastic response of the pulmonary wall in the region of their experimentally produced healed pulmonary infarcts in rabbits and dogs, failed to reach

a/

a conclusion - whether the response of epithelial hyperplasia was an attempt at regeneration of lung tissue. My experimental results, however, revealed definite evidence of regeneration of lung tissue by bronchial budding and alveolar formation which was initiated by the hyperplastic and proliferative activity of pulmonary epithelium from the early phase of repair in both embolically and chemically produced infarcts of lung in cats and rabbits, respectively. This conclusion is supported by a great number of examples illustrated in the descriptive part of this section, which also indicates that regeneration of lung tissue in this respect, is similar to that seen in the healing of lung wounds, and that regeneration of lung alveoli is within the capacity even of a congested lung where infarction was produced embolically with subsequent occlusion of the pulmonary vein in cats.

SUMMARY.

Haemorrhagic and anaemic infarcts were produced in the lung of cats and rabbits, respectively, by injecting liquid poly-vinyl acetate (PVA) as emboli directly into the pulmonary artery/

artery with subsequent occlusion of the vein in cats and by a single sublethal intravenous dose of tetracholorodifluoroethane (TCF) in rabbits.

The reparative phase of the lesions thus produced, has been studied and described.

Microscopic sections of the organised area of the pulmonary infarcts revealed regeneration of pulmonary tissue at the alveolar and bronchial levels to replace the lesion almost completely in 3 - 4 months after the original injury.

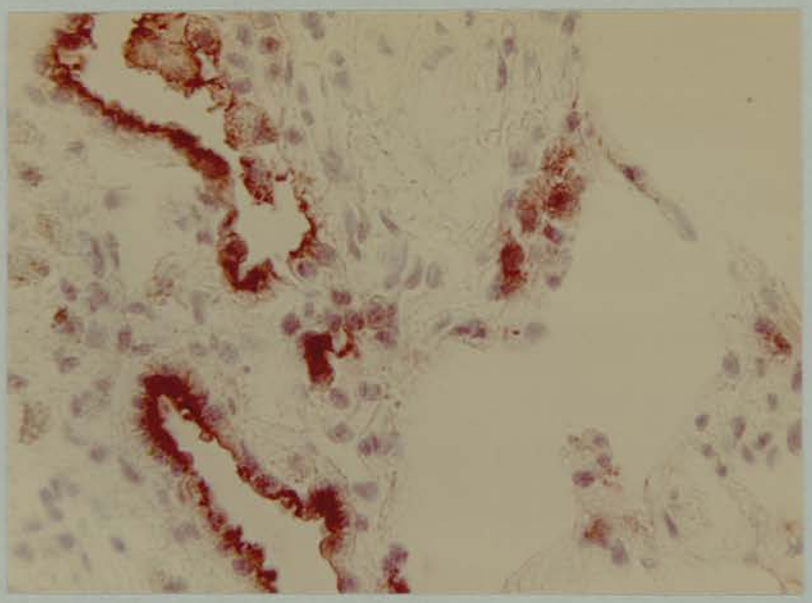

Fig.279 (Rabbit 18.) : 15 days old pulmonary infarct. Alkaline phosphatase reaction. Very mild activity in the lining cells of the bronchial buds. x 480.

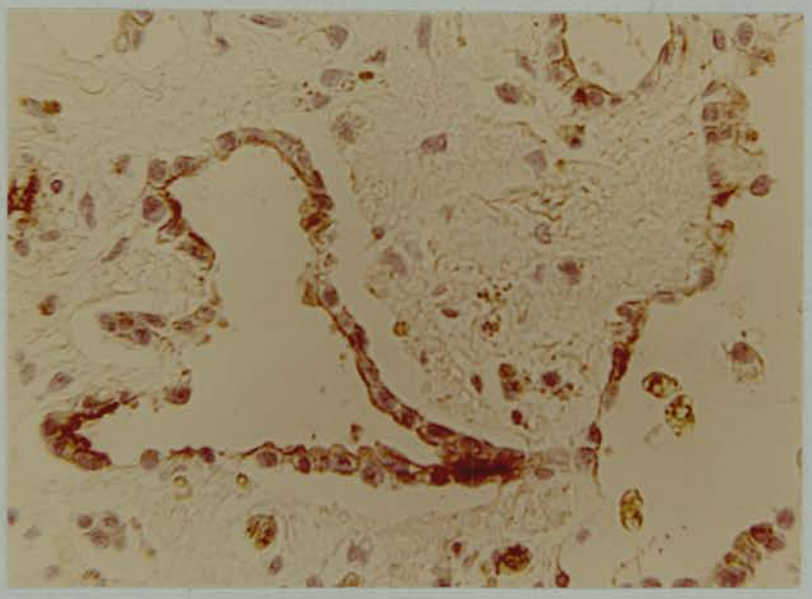

Fig.280 (Rabbit 22.) : 30 days old pulmonary infarct. Very mild alkaline phosphatase reaction in the epithelium of bronchial buds. x 450.

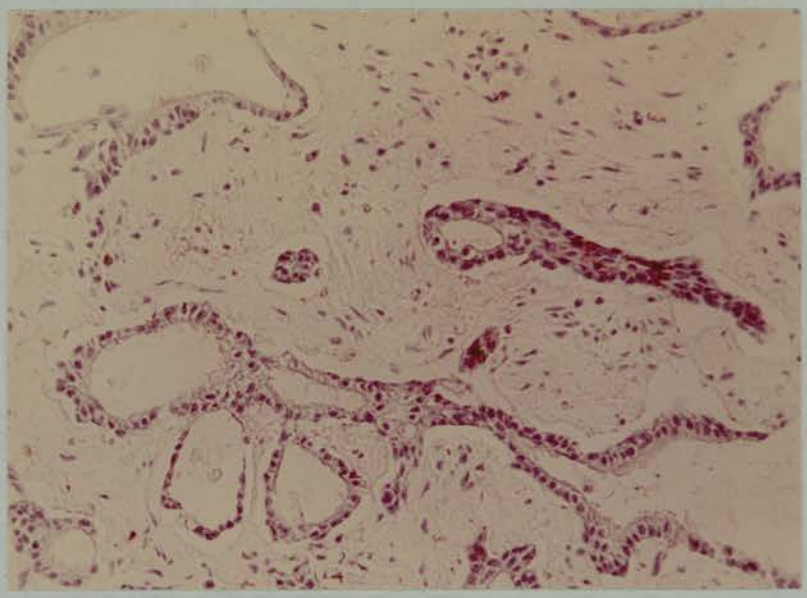

Fig.281 (Rabbit 24.) : 60 days old pulmonary infarct. Bronchial buds showing very weak alkaline phosphatase reaction in their lining cells. x 195.

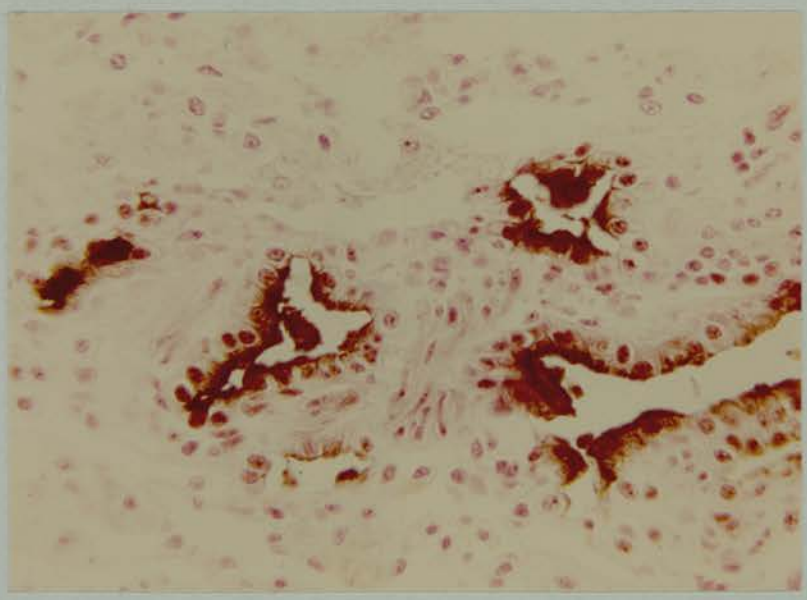

Fig.282 (Rabbit 19.) : 15 days old pulmonary infarct showing fairly strong acid phosphatase reaction in the growing bronchial buds. x 475.

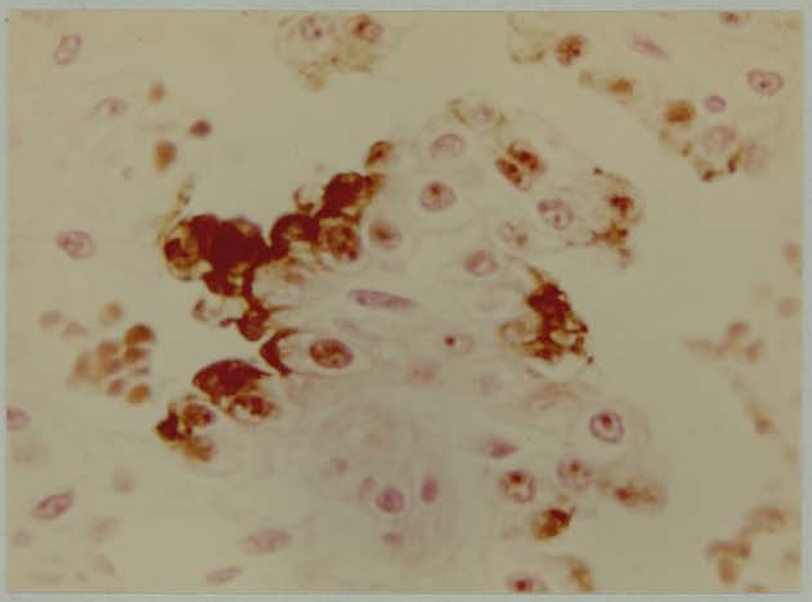

Fig.283 (Rabbit 19.) : 15 days old pulmonary infarct. The swollen alveolar lining cells show strong acid phosphatase reaction. x 1000.

Fig.284 (Rabbit 19.) : 15 days old pulmonary infarct. Mild esterase activity in the lining cells of bronchial buds. x 475.

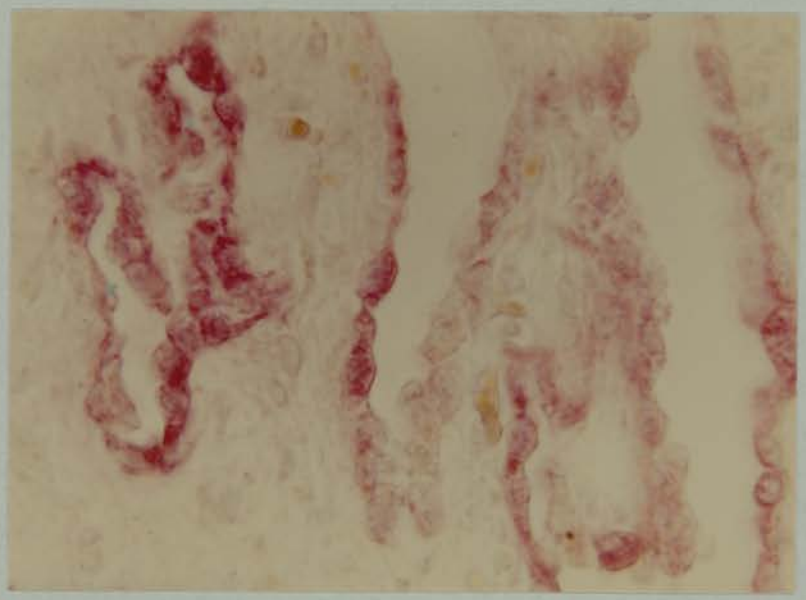

Fig.285 (Rabbit 22.) : 30 days old pulmonary infarct. Mild esterase reaction in the lining epithelium of bronchial buds. x 725.

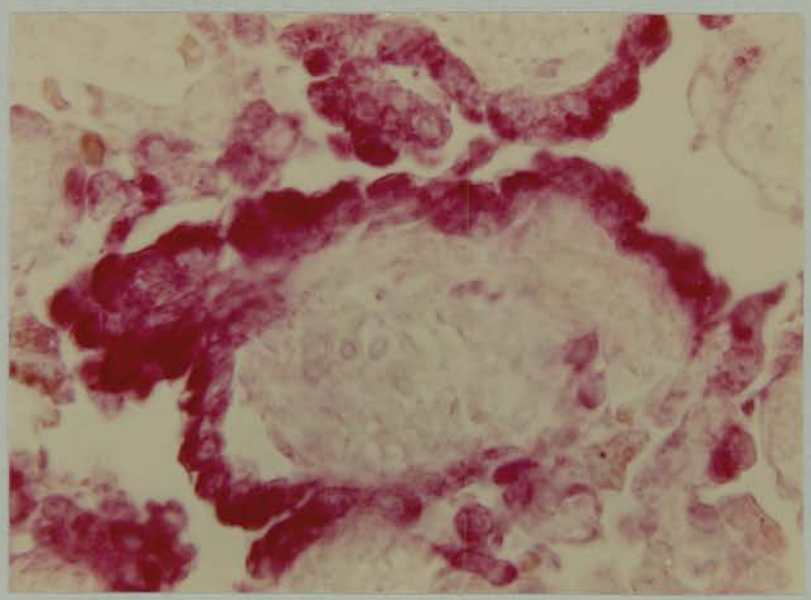

Fig.286 (Rabbit 19.) : 15 days old pulmonary infarct. The proliferating swollen lining cells of alveoli show strong esterase reaction. x 700.

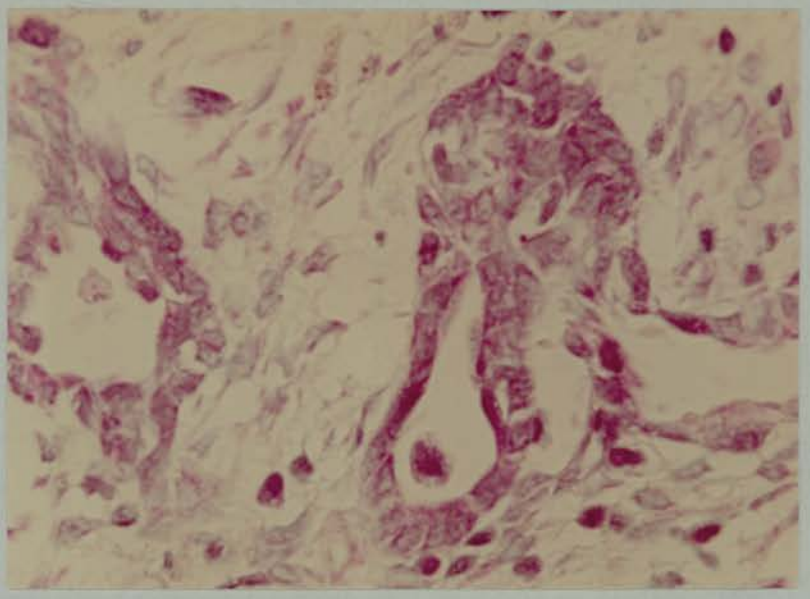

Fig.287 (Rabbit 19.) : 15 days old pulmonary infarct. The epithelial cells of bronchial buds show mild activities for R.N.A. & D.N.A. x 750.

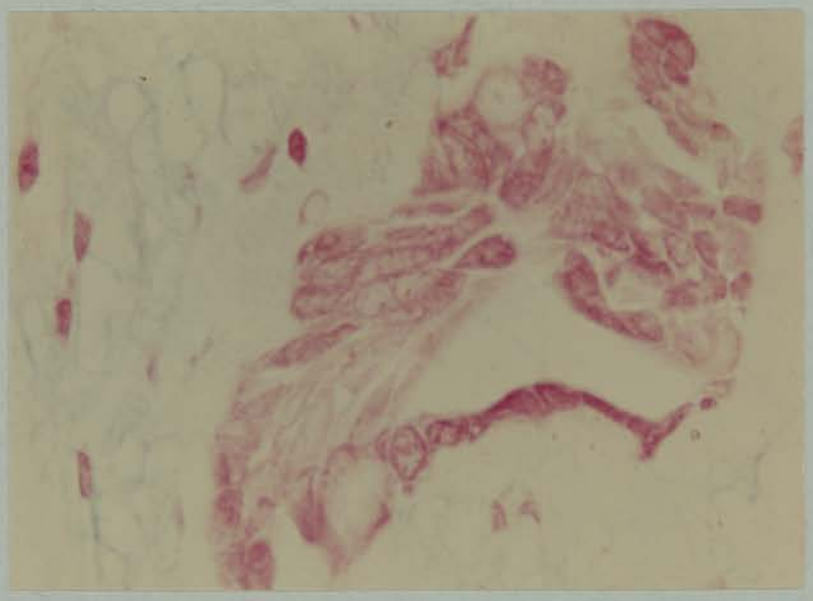

Fig.288 (Rabbit 22.) : 30 days old pulmonary infarct. The epithelial cells of the bronchial bud is negative to alcian blue but the surrounding area of fibroblastic activity is giving positive staining reaction for alcian blue. x 1000.

PART V

GENERAL DISCUSSION

Though perhaps not generally appreciated, the reparative capacity of mammalian lung is not limited to the formation of scar tissue. Montgomery reported that in lung injuries in cats, the scar was gradually transformed into aerating pulmonary alveoli; he thought that this took place partly by bronchial budding and partly by the formation of new alveoli in the scar. He obtained similar though less differentiated results in mice after thermal injury (unpublished). So far as the human subject is concerned, no comparable information is available for obvious reasons; in successful surgical cases no tissue is available and in the fatal post-operative cases, repair is not established. There is, of course, no lack of material from inflammatory and pneumonic conditions where, in resolution, the appearances of alveoli lined by cubical cells and of the organisation of fibrin are well known. The significance of these findings is not always clear and there are no accounts known to me of the regeneration of bronchi in human lung. Similarly, there are several aspects of pulmonary/

pulmonary repair in animals that require further elucidation. Accordingly, the work recorded in this thesis was undertaken with two main objects in view: first, to examine further the repair of lung in human lesions and, secondly, to study the finer details of lung regeneration in animals, particularly with reference to the relative importance of bronchial and alveolar epithelium.

So far as human material is concerned, an examination has been made of tissues from two groups of cases which might be expected to show regenerative changes, namely, bronchiectasis and pulmonary infarcts. The bronchiectatic material has been selected mainly from excised lungs of young patients whose lesions may be said to be primary in the sense that their bronchiectasis was not due to bronchial obstruction from neoplasm or from tuberculous glands. They showed the characteristic appearance of bronchial dilatation of bronchial walls thickened from lymphoid hyperplasia and inflammatory infiltration of the submucosa usually with preservation of the bronchial epithelium. This type of lesion is now believed to be related with infection by adenoviruses/

viruses, partly because these viruses have been recovered from the patients and partly on the analogy of similar lesions in calves where the virus has been isolated and is believed to be directly responsible (Jarrett, 1954).

Microscopic examination of the human material displays a most impressive and active reparative process. Bronchial buds can be seen to spring abundantly from the dilated bronchi and from neighbouring bronchi not so severely affected and to penetrate actively through the inflammed peribronchial tissues. New airways lined by cubical cells continuous with the bronchial lumen ramify throughout the granulation tissue and the lymphoid follicles: accordingly, pneumonic patches occur in the lung and there also organisation is a feature but the main activity seems to be by bronchial budding. The change is so well-established that it seems almost to have developed in parallel with the bronchiectatic lesion, as though the stimulus of the peribronchial inflammation had been immediately effective in provoking bronchial regeneration.

So far as my examinations are concerned, no other human lung lesion shows comparable activity/

activity. Some tuberculous lungs demonstrate bronchial budding to some extent but the picture is usually modified by caseation which stimulates mainly a growth of fibrous tissue, a barrier of mature collagenous tissue which gives the impression of encroaching slowly upon the caseous mass. In those circumstances bronchial budding is absent or only trivial in extent. Reports of repair in tuberculosis treated by chemotherapy have been discussed by Nagai & Fujimaki, 1958, who has described some cases in detail but bronchial budding and alveolar regeneration do not appear to be recognised either in his own work or in the literature discussed. It has, of course, to be borne in mind that most of the bronchiectatic lungs which I have examined are from relatively young patients in whom growth of all kinds may be presumed to be prominent. Nevertheless, it is difficult to believe that this is entirely a factor of age, particularly in view of the reparative changes in human pulmonary infarcts.

As has been pointed out earlier, a search for reparative process in infarcts of lung involved the examination of a very large number (851 cases of pulmonary infarcts in a series of 16137 consecutive autopsies) of records in the/

the Royal Infirmary of Edinburgh. This took a considerable time but showed beyond doubt that the majority of pulmonary infarcts occur in patients with cardiac conditions, with pulmonary congestion, and that few survive long enough for reactive change to occur. Of the survivors, all were adults of middle or old age. Consequently the well-established reparative changes shown in those tissues clearly discredit the view that regeneration of lung is a feature of youth. The eleven examples chosen for study are of remarkable interest and establish undisputably that the organisation of a pulmonary infarct is not confined to encapsulation or to its absorption as some text-books say, but may progress to a true morphological and functional restoration of lung tissue. Naturally, this is associated with cicatrical organisation with fibroblastic proliferation and vascularization. The infarcts studied were all bland and accordingly there was no problem of infection. Thus the bronchial buds leading to new alveolar formation was clearly shown. Low power photomicrographs emphasise that the direction of this process was towards the infarct, a directional impulse towards consolidated tissue, a point/

point to which Cameron has directed attention in his 'Pathology of the cell' (1952).

Of the unusual features of these lesions, attention may be directed to Case (Figs. 31, 32 & 33) No. 11 which shows the unusual feature of the phagocytosis of red blood corpuscles by the epithelium of the bronchial buds. As the new air passages penetrate the recent haemorrhage of the infarct, actual ingestion of the red blood corpuscles takes place by the epithelial cells of the air passages. So far as is known, phagocytosis is not a feature of the bronchial epithelium but it is a well-established property of alveolar epithelium. In the sections the epithelium showing this property lines, what are to all intents and purposes, bronchial buds; the passages are continuous with the parent bronchi and only a microscopic distance from them; the epithelial cells are plump cubical cells with all the physical attributes of bronchial bud epithelium. This spontaneous demonstration of phagocytic property by cells which, however regarded, are at least transitional between bronchial and alveolar cells, is additional evidence in support of the contention of this thesis, that the re-formation of/

of lung alveoli is due essentially to bronchial outgrowths.

The studies on wounds of lung are to some extent a confirmation of the earlier work of Montgomery. They show the bronchial budding which he described and the formation of new alveoli lined in part or wholly by cubical cells. It is submitted, however, that from the diversity of their nature, the studies recorded here show that the regenerative changes are due primarily to bronchial activity. All transitions can be seen between the bronchial buds and the air spaces and study of the sections has led me to doubt whether alveolar spaces ever reform except by bronchial intervention. Admittedly, collapsed alveoli may re-open, sometimes with a cubical lining, but where there is true organisation of scar tissue or fibrin, air spaces appear to acquire their lining cells from pre-existing bronchial epithelium. The bronchial cells elongate and extend into cracks and fissures in the scar tissue so converting them into air sacs: naturally, the highly-vascular nature of organising tissue in lung makes this readily feasible.

In support of this contention, there is to/

to be noted first themorphological similarity between the alveolar cubical cells and those of the bronchial buds. The similarity is further demonstrated by their similar behaviours to enzyme staining methods. Bronchial epithelium normally shows R.N.A. and D.N.A. enzymic activity to a moderate degree and after injury all gradation can be seen between this and the fully-developed activity of the cubical cells of bronchial buds and alveoli. This is a continuous process physically and functionally in the animal lungs. Thirdly, there is the confirmatory evidence of the uptake of radio-active sulphur, normally a property of bronchial epithelium and in repair, found also in the bronchial buds.

King believed that cicatrical fixation of pulmonary tissue was the principal predisposing factor to bronchial hyperplasia. Peterson and associates (1949) stressed tne occurence of the epithelial proliferation in their cases of bronchiectasis, bronchitis or focal fibrosis. In my studies hyperplasia of pulmonary epithelium was an early reaction which was observed to occur long before a fixing tissue was formed in the healing lung of experimental wounds and infarcts./

fa[illegible]cts.

Some authors considered the type of epithelial proliferation as neoplasia. Prior and Jones (1952) and Prior (1953) believed that there was a similarity to bronchial adenoma. Raeburn and Spencer (1953) and Zatuchni and associates (1953) considered foci of bronchiolar hyperplasia as probable sites for the development of bronchiolar carcinoma. In my experimental series epithelial hyperplasia was a main feature in the reparative process of both experimental wounds and infarcts of the lung. This was a very early phenomenon and continued to occur even in 4 months-old lesions. But, as has been previously mentioned, there was no evidence of unrestricted growth to consider the lesions malignant nor was there any indication of anaplasia or invasion in these hyperplastic cells in a single instance.

It is admitted that this evidence is corroborative rather than a final proof; because two things look alike, they are not necessarily the same. Alveolar epithelium regenerating in situ may be histologically indistinguishable from bronchial bud epithelium though the present experiments suggest that this is unlikely. All the/

The evidence of many sections suggests that new alveoli form in organising lung tissue from the activity of pre-existing bronchi.

This conclusion raises again the vexed question of the significance of alveolar cubical lining cells, seen so often in pulmonary pathology. It appears that this may arise in two ways. There is no doubt that it may occur in the process of re-formation of lung, regeneration as part of organisation: this has been seen in wound and infarcts experimentally and in repair in human tissues. But undoubtedly it occurs also in alveoli damaged in a variety of pathological processes: in inflammation and in collapse where in the process of resolution or re-expansion, cubical cells form. In these circumstances, the cells are not of bronchial origin: they are alveolar lining cells which for some reason have become plump and swollen. Perhaps it may indicate protein synthesis in the cell cytoplasm but of this no evidence at present is seen. Many writers refer to 'foetalisation' in an attempt to describe this change, having in mind the appearance of foetal lung. It may be that under conditions of imperfect aeration a cubical form is/

is the optimum shape of cell. Alternatively, the cubical cells may be potential phagocytes, ready to become free in the alveolar spaces. These are no more than hypothesis to emphasise the point that it is not the contention of this thesis that all alveolar cubical cells are regenerative in nature. Regenerative cells are primarily bronchial in nature and may in fact have no other origin.

One of the most interesting features in the regenerative process of lung tissue is the direction of the bronchial buds towards the injured part. Presumably the stimulus to initiate this directional impulse to the regenerate for the restoration of the lost part is derived mainly from the wound itself. This initiation to regenerate the lost part is the most crucial step in the sequence of events of normal regeneration and is believed to be induced, at least in lower creatures, by a 'wound factor' or 'regeneration-promoting factor' (RPM), formed immediately a part is lost. It is produced extremely locally and probably only from cells actually damaged. (Needham, 1952). Wigglesworth (1937), from experiments of insect epidermis, suggested that an activating substance liberated/

liberated by wounding starts migration of epithelial cells.

The generally accepted view of the sequence of events of regeneration consists of two phases: regressive and progressive. The former includes, in order, (1) wound-closure, (2) demolition of damaged cells and defence, and (3) differentiation of cells to provide new tissue for the progressive phase. The latter includes (1) formation of the regeneration-bud or blastoma, (2) growth of the regeneration-bud, and (3) differentiation of the young regenerate followed by recovery of function. The morphologic evolution of repair of lung tissue in experimental wounds has been observed also to behave accordingly. There is, of course, considerable overlap between successive stages in different regions of the area of regeneration. Here also the reactive process starts with the formation of blood-clot in the wound-gap to prevent excessive bleeding as well as to provisionally close and protect the wound. This is followed by the stage of demolition by the activity of scavenging cells, macrophages, etc., which remove the debris of the wound to prepare the ground for the progressive or true regenerative phase. This is/

is accomplished on the one hand by fibroblastic reaction leading to granulation tissue formation, and on the other, by epithelial cell proliferation to give rise to the formation of bronchial buds. Both reactions go on partly simultaneously and partly in succession in the remaining tissue. The fibroblastic reaction proceeds and organises the area of the wound and the proliferative activity of bronchial epithelium gives rise to bronchial buddings which penetrate the organised area to re-aerate the part. It is probable at this stage that, in addition to the traumatic stimulus, the presence of fibrin also acts as a stimulating factor for cellular hyperplasia of bronchial epithelium. In support of this view are the facts that these changes are shown by the epithelium of bronchi and alveoli or portions of alveoli nearest to the blood-clot of the wound and those alveolar cells beneath the pleura to which a varying quantity of fibrin adheres.

As soon as a new channel buds off from the parent trunk it fills up with atmospheric air and becomes exposed to rhythmic variations in internal pressure which transmits an additional penetrative/

penetrative power, over and above the directional impulse, through the bronchial buds in their long axis. This penetrative activity further enhanced by the regular expansion and contraction of the bronchial buds which facilitate their passage through unexpanded lung and aid splitting of denser tissue. These are probably the reasons, viz., the directional impulse received from the stimulus of wounding, the penetrative power transmitted through the bronchial buds by the internal pressure from the parent stem, and the regular expansion and contraction of the bud itself, why the bronchial bud is projected towards the wound.

In the final stage, differentiation of the regenerates takes place to recover the functional state of the damaged part of the lung. With all probability this may occur in the following manner: the developing bronchi become new alveoli which are lined by cubical cells. These cells resemble those of foetal lung, but ultimately flatten out to assume the form of the lining cells of normal alveoli. When many air spaces are formed in the regenerated area the septa between them begins to thin out and blood vessels get into them.

It/

It is not known whether cubical alveolar cells are capable of gaseous exchange or whether this function is limited to normal alveolar walls. However, the relationship of the cubical cells to the pulmonary alveolar capillaries does not exclude the possibility of function; it is impossible to say more from histological studies.

Defects in the bronchial lining are rapidly filled in by migrating and dividing epithelium as in the case of trachea.

GENERAL SUMMARY

Reparative changes and regeneration of lung tissue have been studied in human material of two types: haemorrhagic infarcts, collected from autopsy material; and inflammatory lesions - tuberculous and non-tuberculous, removed surgically. In both groups of cases regeneration of lung tissue was found with new bronchial and alveolar formation.

As a basis for later works on experimental study of lung repair and also to determine whether the application of histochemical methods would be of value, healing of tracheal mucosa after/

after curettage has been studied in rats. It was observed that repair of the injured mucosa took place by migration of flattened cells derived from the marginal epithelium and exposed neck of submucosal glands and regeneration was complete by the formation of normal tracheal epithelium in 33 - 45 days after the original injury. Histochemical study of the regenerating epithelium proved only of limited value.

Repair of experimental wounds, produced by the excision of a triangular wedge of lung tissue in adult cat, has been studied. Active regeneration of lung tissue with formation of bronchi and new alveoli was observed until the whole area of the wound was transformed into functional lung in 3 - 4 months.

Theuptake of injected ^{35}S sulphate by the pulmonary epithelium in experimental healing wound of lung has been studied in cats by means of autoradiography. The lining cubical cells of newly-formed bronchial buds and alveoli in the regenerating area of the lung, together with the bronchial epithelium, were observed to take up radioactive sulphur.

Reparative changes of experimental pulmonary infarcts, produced embolically by introducing/

introducing liquid polyvinyl acetate (PVA) in thepulmonary artery in cats with subsequent occlusion of the pulmonary vein in one lung and chemically by intravenous injection of tetracholrodifluoroethane (TCF) in rabbits, have been studied. Regeneration of lung tissue in alveolar and bronchial levels was found to develop during healing of the above lesions and almost the whole infarcted area was observed to regenerate in 4 months after operation.

The significance of the findings obtained from the above observational and experimental studies in relation to regeneration of lung has been discussed.

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APPENDICES

APPENDIX I

HISTOCHEMICAL STAINING METHODS

I. ALKALINE PHOSPHATASE

Fixation: Thin blocks of tissue should be fixed according to Gomori in 2 or 3 changes of cold absolute acetone at 4°C for 24 - 48 hours. The tissue is best embedded in the following ways:

1. Transfer the blocks progressively at ½ hour intervals to absolute ethanol, absolute ethanol-ether with 1 or 2 changes, and thence to 1 percent celloidin.
2. Drain excess of celloidin and harden in chloroform.
3. Clear in benzene.
4. Embed in paraffin wax, avoiding prolonged exposure to the high temperature of the wax-bath.
5. Cut sections at 5u and mount on clean slides. (Albunised or non-albunised).
6. Dry the slides for 3 hours at 37°C.
7. Store at 4° until ready for incubation.

Reagents:

1. 0.1M-Vernol acetate buffer pH 9.16 (Pearse/

(Pearse, p 408). Stock solution: 9.714 g. Na-acetate $3H_2O$ + 714 g. Na-barbiturate in CO_2-free distilled water, made up to 500 ml. 5 ml. of this solution + 2 ml. of 8.5 per cent NaCl (may be omitted) treated with X ml. of 0.1N HCl and (18-X) ml. water.

X ml. 0.1N HCl	pH.	X ml. 0.1N HCl	pH	X ml. 0.1N HCl	pH
16.0	2.62	9.0	4.93	4.0	7.66
15.0	3.20	8.0	5.32	3.0	7.90
14.0	3.62	7.0	6.12	2.0	8.18
13.0	3.88	6.5	6.75	1.0	8.55
12.0	4.13	6.0	6.99	0.75	6.68
11.0	4.33	5.5	7.25	0.5	8.9
10.0	4.66	5.0	7.42	0.25	9.16

2. Na-alpha-naphthyl phosphate.
3. Diazotates of the base 5-chloro-O-toluidine (C.I.C.).

Method:

1. Dissolve 30 - 40 mg. of Na-alpha-naphthyl phosphate in 90 ml. of buffer.

2./

2. Add 30 mg. of diazotates of base 5-chloro-O-tuluidine.
3. This is filtered into a staining jar full of mounted sections which have been brought to water in the usual way.
4. Leave at room temperature for some hours or overnight.
5. Counterstain with haematoxylin; differentiate in acid alcohol; mount in glycerin jelly.

Results:

Alkaline phosphatase activity is shown by brick-red or dark-reddish-brown; nuclei - blue.

ACID PHOSPHATASE

This is the least consistent method but may work very well on occasion. It is to be used exactly as described on page 193 of Gomori's (Microscopic Histochemistry'.

Fixation:

Fix 2 mm. blocks of tissue in anhydrous acetone at $4^{o}C$ for 24 hours. Complete dehydration in three further changes of acetone at room temperature/

perature, 30 minutes changes each or in 100 per cent alcohol for 24 hours. It should be cleared next by acetone-benzene (or cedar oil) mixture for 30 minutes followed by 2 thirty minutes changes of benzene. Paraffin infiltration should be done by 15 to 30 minutes in vacuo or ordinary infiltration not more than 3 hours.

Buffer:

Molar acetate buffer pH 4.7	5 c.c.
Lead nitrate - $Pb(NO_3)_2$-5% - 100 mg.	2 c.c.
Distilled water	87 c.c.
Sodium glycerophosphate (2% - 6 c.c. 120 mg.	6 c.c.
Total volume	100 c.c.

Method:

1. Bring 5 - 6u sections to water as usual.
2. Rinse in distilled water.
3. Incubate in the lead nitrate sodium glycerophosphate solution at 37°C for 1½ - 24 hours.
4. Rinse in distilled water.
5. Then to 2 - 3 per cent acetic acid.
6. Wash thoroughly in distilled water.
7. Immerse for 1 or 2 minutes in 1:40 or 1:50 dilution of yellow ammonium sulphide.

8./

8. Wash thoroughly in tap water. Counterstain as desired, either with haematoxylin and eosin or basic fuchsin.

Result:

Sites of acid phosphatase activity are shown by dark brown deposit of lead sulphide.

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(3) NON-SPECIFIC ESTERASE

Fixation:

Blocks of tissue are fixed in cold acetone at 4°C for 24 hours, 2 or 3 changes.

Reagents:

1. Substrate solution : 1% Brenthol. AS-acetate in equal parts of acetone and alcohol. For preparation of Brenthol AS-acetate see Pearse - page 462.

   Dissolve 5 g 2-hydroxy-3-naphtholic (NaphtholAS-I.C.I.) in 10 ml. dry pyridine with 20 ml. acetic anhydride in addition. Heat under reflux condenser for 1 hour. Pour into cold water, filter off the pasty product and dry. Recrystallize from ethanol (containing a little charcoal) to obtain the/

the acetate as a cream-coloured powder (m.p. 160 - 161°C).

2. Propylene glycol.
3. Any stable diazoate, best in Diazo Garnet (Gurr).
4. 0.2M phosphate buffer pH. 7 - 7.2.

0.2M phosphate buffer:

Soln.A : Monobasic Na-phosphate, 2.76 g. in 100 ml. or Pot. dihydrogen phosphate 3.13 g in 100 ml.

Soln. B : Dibasic Na-phosphate, 5.36 g. ($Na_2 2HPO_4 . 7H_2O$), or $Na_2HPO_4 . 12H_2O$, 7.6 g. in 100 ml.

A (ml)	B (ml)	pH	A (ml)	B (ml)	pH
90	10	5.9	33	67	7.1
85	15	6.1	23	77	7.3
77	23	6.3	19	81	7.4
68	32	6.5	16	84	7.5
57	43	6.7	10	90	7.7
45	55	6.9			

Method:

1. Add 2 ml. of the substrate solution to 25 ml. of glycol. Dilute slowly to 90/

90 ml. with distilled water. Add 10ml. of buffer and about 50 mg. of Diazo Garnet.

2. Filter into a staining jar which contains the sections already brought to water as for phosphatase and proceed thereafter in similar way.
3. Leave at room temperature for some hours, even up to 14 hours, or incubate at 17 - 22°C for 20 - 30 minutes.
4. Wash in running water for 1 minute. (Omitted).
5. Counterstain with Mayer's haemalum, 2 - 3 minutes.
6. Wash blue in dilute ammonia.
7. Wash and mount in glycerine jelly. (Differentiation omitted).

Result:

A particulate of azo dye is deposited at sites of esterase activity.

---

(4) RNA and DNA

Tissue is fixed in modified Baker's fluid for 24 hours at room temperature or short fixation in 10% formalin at ph 7.0± 0.2, 4 - 16 hrs.

Process/

Process:

Dehydrate in alcohol for 6 - 12 hours by 2 - 3 changes and clear in xylol. Impregnate with paraffin and embed. Cut section at 5 - 6u and put the slides in an incubator overnight. Stain.

Method:

KURNICK'S PLASMA CELL STAIN FOR RNA-DNA.

1. Staining solution:

   (a) Pyronin Y (Gurr) - chloroform extracted - 2% aqueous 12.5 ml.

   (b) Methyl green - chloroform extracted - 2% aqueous - 7.5 ml.

   (c) Distilled water - 30.0ml.

   Mixed the three and ready for use.

2. Immerse slides for 6 minutes in the staining solution.
3. Blot with filter paper.
4. Immerse slides in 2 changes in N-butyl-alcohol, 5 minutes in each.
5. Clear in xylol. Mount in DPX.

Result:

RNA - pinkish cytoplasm

DNA - light green nuclei

Modified Baker's fluid.

Formalin - 125 cc.

Sat.soln. & NaCl - 27 cc.

$CaCO_3$ 1 g.

Tap water to 1000cc.

ALCIAN BLUE.

Fixation:

10% formol-saline for 48 hours.

Methods:

1. Bring sections to water.
2. Stain in a freshly prepared 1% aqueous solution of alcian blue for 45 - 60 seconds. If longer time is allowed other tissues will take the stain.
3. Rinse in distilled water.
4. Stain in 1% neutral red for 2 - 3 minutes.
5. Rapidly dehydrate in alcohol, clear inxylene and mount in DPX.

Result:

Mucopolysaccharides - clear blue green.

Nuclei - dark red.

APPENDIX II

AUTORADIOGRAPHIC TECHNIQUE

(Doniach & Pelc, 1950)

Embedding:

Blocks of tissue fixed in 10% formol-saline for 48 hours. (Bouin's solution may be used).

Dehydrate/

Dehydrate: 80% alcohol ..... 1 hr.
90% " ..... 1 hr.
95% " ..... 1 hr.
Abs. " ..... 2 hrs.
2 changes.

Chloroform - overnight.

Wax - 35, 30, 30 and 45 minutes then embed in paraffin in vacuo (2 hrs. 20 mins.)

Staining:

1. The slides on which the sections are mounted should preferably be 'subbed' in a 'subbing solution' to ensure good wet-adhesion of the emulsion film when the autoradiographs are processed.
2. Cut sections at 5 - 6u and mount on 'subbed' slide.
3. Dewax and stain (a) Ehrlich's haematoxylin for routine examination.
(b) Alcian blue and PAS for mucopolysaccharides.

Exposure:

Until the fixing stage, the entire processing of autoradiography should be carried out in an ideal dark room under proper safelight. (Safelight used - Kodak Safelight 'Wratten' Series/

Series I (red), using 25-watt bulb).

1. With a sharp blade cut through the emulsion layer (Kodak Autoradiographic Stripping Plates AR 10) around an area sufficient to cover the whole of the specimen, with a margin at least a quarter of an inch wide all round.
2. With the tip of the blade, strip the section of composite layer from the glass plate, turn it over, and throw it on to the surface of the water (preferably in a large bowl) so that the emulsion side is facing downwards. The water must be completely grease-free distilled water. Cleanliness of the water particularly of the surface is most important since any floating particles will tend to be trapped between the emulsion and the specimen.
3. Mount film on slide, emulsion side direct in contact with sections.
4. The specimen, with the superimposed emulsion, should be dried in a steam of cool air (a hair-drier can be used) and then placed in a light-tight box for exposure. The box should be stored/

stored at a temperature not exceeding 70°F (3 - 21°C) in a light-tight steel cabinet in the dark room.

Developing and Fixing:

1. Prepare developer from stock solution "Dolmi" D170. Dip the prepared slide in the developer for 5 - 10 minutes.
2. To neutralise any excess developer rinse the slides thoroughly for about 20 seconds in two changes in water at 20°C.
3. Fix at 18°C in a solution of Kodak Acid Fixer Powder for twice the time taken in the developer. (Kodak Acid Fixer Powder to be dissolved in 1870 cc. of distilled water - filter and use). 10 minutes.
4. Next the preparation is washed in running water for 10 - 15 minutes.
5. Dehydrate as follows: In 50% alcohol - 30 minutes. Methylated spirit - 30 min. (2 changes). Absolute alcohol - 30 min.
6. Clear in phenol-xylol overnight.
7. Mount in DPX.

Staining for autoradiography:

Haematoxylin and Eosin:

1./

1. Section to water.
2. Stain with Ehrlich's haematoxylin for $1\frac{1}{2}$ - 2 hours at 40 - 50°C.
3. Differentiate individually in acid alcohol.
4. Blue in running water for 1 - 2 hours. (The stain should be left a bit darker than the normal process since a certain amount of bleaching occurs in the 'Dolmi' developer.
5. Take up to alcohol 95%.
6. Counterstain with alcoholic eosin for 2 - 3 minutes.
7. 2 changes in absolute alcohol.
8. Direct to autoradiographic processing.

PAS:

1. Bring sections down to water.
2. In periodic acid - 5 minutes.
3. Immerse in Schiff's solution - 10 - 20 mins.
4. Three 2 min. changes in sulphite-reducing-bath.
5. Wash in running water - 10 mins.
6. Counterstaining was done by Einarson's gallocyanin - chrom alum.
7. After counterstain direct to autoradiographic/

graphic processing.

Sulphite-reducing-bath : 5 ml. normal HCl

6 ml. 10% Na-metasulphite.

Water up to 100 ml.

Counterstaining: (1) To 100 ml. of 5% aqueous solution of chrom alum is added to 0.15 g. of gallocyanin.

(2) Shake well, bring slowly to boil, and allow to boil for 5 mins.

(3) Cool, filter, and make up the volume of the filtrate to 100 ml. by adding distilled water through the filter paper.

Method: (1) Stain the section in gallocyanin-chrom alum for 48 hours at room temperature.

(2) Wash in water for a few seconds.

(Normal counterstain for PAS in haematoxyline which is bleached by the developer in radiographic technique).

Result:

Mucin is pink, nuclei - blue.

Periodic/

Periodic acid:

0.5%

Schiff's reagent:

1. Dissolve 1 g. basic fuchsin in boiling 200 ml. distilled water.
2. Cool to 60°C and filter.
3. Add 20 ml. norm 1 HCl.
4. Cool to 20°C.
5. Add 2 g. of Na-bisulphite.
6. Leave for 48 hours and use.

Alcian Blue:

1. Section to water.
2. Stain in 1% freshly prepared aqueous alcian blue - 45 - 60 secs.
3. Wash in water.
4. Counterstain with Feulgen technique:
   (a) Section in water.
   (b) Hydrolyse 10 mins. at 60°C in normal HCl.
   (c) Followed by immersion in Schiff's reagent 1½ - 2 hours.
   (d) Three 2 minute changes in sulphite-reducing-bath.
5. Wash 10 mins in water.
6. Direct to autoradiographic processing.
   (Ordinary counterstain,- aqueous neutral red/

red is bleached in 'Dolmi' developer).

Result:

Mucin - bluish-green; Nuclei - pink to purple.

Subbing solution:

Potassium bichromate	100 g.
Sulphuric acid (Conc.)	100 c.c.
Water to make	1000 c.c.

Dissolve the bichromate in the water, then add the sulphuric acid slowly to the cold solution with constant stirring.

Kodak Formula:

D170 - stock solution.	.....	
Sodium sulphite (Cryst.)	.....	50 g.
(or anhydrous).	.....	(25 g.)
Potassium bromide	.....	1 g.
Water to	.....	200 c.c.

For use dilute the above 200 c.c. of stock solution with waterto make 1000 c.c. and dissolve in this 4.5 g. of 'Dolmi' developing agent. The diluted solution does not keep well and should be made up as required.

Ehrilch's Haematoxylin:
(Acid)

Haematoxylin	2 g.
Absolute alcohol	100 ml.

Acetic/

Acetic acid	10 ml.
Glycerol	100 ml.
2.5% aq. soln. potash alum	100 ml.

The above solution must be allowed to mature naturally, in sunlight, and use at about 50°C till tissue is over-stained. Differentiate with acid-alcohol (70% alcohol and 6% HCl).

Autoradiographic plates:

The plates are packed in 3 groups of 4; in each pack they are placed in 2 pairs, face to face, the middle ones which are back to back being clipped together with a cardboard end-piece. If the plates have been stored at about 2°C it will take a few minutes for edges of the cut sections to begin to curl up, they come off much more easily if thebox has been out of the 'fridge' for a little time before starting. (Described as Kodak Stripping Film on 4¾ x 6½ plates for autoradiography, boxes of twelve.)

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